

JC649

U.S. PTO

# UTILITY PATENT APPLICATION TRANSMITTAL

Title of Invention \*

METHODS AND COMPOSITIONS FOR INHIBITION  
OF ANGIOGENESIS

Named Inventor(s)

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under 37 CFR 1.53(b))

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## APPLICATION ELEMENTS

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3. ☒ Drawings Total Sheets: 7
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**[Note Box 5 Below]**
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Signed statement attached  
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The entire disclosure of the prior application, from  
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7. ☐ Nucleotide and/or Amino Acid Sequence  
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## ACCOMPANYING APPLICATION PARTS

8. ☐ Assignment Papers (cover sheet &  
document(s))
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(when there is an assignee)  
☐ Power of Attorney by assignee
10. ☐ English Translation Document (if applicable)
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☐ Statement filed in prior application  
Status still proper and desired
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17. If a **CONTINUING APPLICATION**, check appropriate box and supply the requisite information:
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## **METHODS AND COMPOSITIONS FOR INHIBITION OF ANGIOGENESIS**

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### **Related Applications**

This application claims priority to U.S. provisional application Serial No. 60/028,708, filed November 5, 1996.

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### **Technical Field**

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The present invention relates to methods and compositions for preventing unwanted angiogenesis in a human or animal. More particularly, the present invention relates to a method for preventing unwanted angiogenesis, particularly in angiogenesis dependent or associated diseases, by administration of compounds such as thalidomide and related compounds.

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### **Background of the Invention**

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As used herein, the term "angiogenesis" means the generation of new blood vessels into a tissue or organ. Under normal physiological conditions, humans or animals only undergo angiogenesis in very specific restricted situations. For example, angiogenesis is normally observed in wound healing, fetal and embryonal development and formation of the corpus luteum, endometrium and placenta. The control of angiogenesis is a highly regulated system of angiogenic stimulators and inhibitors.

The control of angiogenesis has been found to be altered in certain disease states and, in many cases, the pathological damage associated with the disease is related to uncontrolled angiogenesis.

5 Both controlled and uncontrolled angiogenesis are  
thought to proceed in a similar manner. Endothelial cells and  
pericytes, surrounded by a basement membrane, form capillary  
blood vessels. Angiogenesis begins with the erosion of the  
basement membrane by enzymes released by endothelial cells and  
leukocytes. The endothelial cells, which line the lumen of blood  
10 vessels, then protrude through the basement membrane.  
Angiogenic stimulants induce the endothelial cells to migrate  
through the eroded basement membrane. The migrating cells  
form a "sprout" off the parent blood vessel, where the endothelial  
cells undergo mitosis and proliferate. The endothelial sprouts  
15 merge with each other to form capillary loops, creating the new  
blood vessel. In the disease state, prevention of angiogenesis  
could avert the damage caused by the invasion of the new  
microvascular system.

20 Persistent, unregulated angiogenesis occurs in a  
multiplicity of disease states, tumor metastasis and abnormal  
growth by endothelial cells and supports the pathological damage  
seen in these conditions. The diverse pathological states created  
due to unregulated angiogenesis have been grouped together as  
angiogenic dependent or angiogenic associated diseases. Therapies  
25 directed at control of the angiogenic processes could lead to the  
abrogation or mitigation of these diseases.

One example of a disease mediated by angiogenesis is  
ocular neovascular disease. This disease is characterized by  
invasion of new blood vessels into the structures of the eye such  
30 as the retina or cornea. It is the most common cause of blindness  
and is involved in approximately twenty eye diseases. In age-  
related macular degeneration, the associated visual problems are  
caused by an ingrowth of chorioidal capillaries through defects in  
Bruch's membrane with proliferation of fibrovascular tissue  
35 beneath the retinal pigment epithelium. Angiogenic damage is

also associated with diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma and retrolental fibroplasia. Other diseases associated with corneal neovascularization include, but are not limited to, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogren's syndrome, acne rosacea, phlyctenulosis, syphilis, *Mycobacteria* infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, *Herpes simplex* infections, *Herpes zoster* infections, protozoan infections, Kaposi's sarcoma, Mooren's ulcer, Terrien's marginal degeneration, marginal keratolysis, rheumatoid arthritis, systemic lupus, polyarteritis, trauma, Wegener's sarcoidosis, Scleritis, Steven's-Johnson disease, radial keratotomy, pemphigoid and corneal graft rejection.

Diseases associated with retinal/choroidal neovascularization include, but are not limited to, diabetic retinopathy, macular degeneration, sickle cell anemia, sarcoid, syphilis, pseudoxanthoma elasticum, Paget's disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus erythematosus, retinopathy of prematurity, Eales' disease, Behcet's disease, infections causing a retinitis or choroiditis, presumed ocular histoplasmosis, Best's disease, myopia, optic pits, Stargardt's disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser complications. Other diseases include, but are not limited to, diseases associated with rubeosis (neovascularization of the angle) and diseases caused by the abnormal proliferation of fibrovascular or fibrous tissue including all forms of proliferative vitreoretinopathy.

Another disease in which angiogenesis is believed to be involved is rheumatoid arthritis. The blood vessels in the synovial lining of the joints undergo angiogenesis. In addition to forming new vascular networks, the endothelial cells release factors and reactive oxygen species that lead to pannus growth and

cartilage destruction. The factors involved in angiogenesis may actively contribute to, and help maintain, the chronically inflamed state of rheumatoid arthritis.

5 Factors associated with angiogenesis may also have a role in osteoarthritis. The activation of the chondrocytes by angiogenic-related factors contributes to the destruction of the joint. At a later stage, the angiogenic factors would promote new bone formation. Therapeutic intervention that prevents the bone  
10 destruction could halt the progress of the disease and provide relief for persons suffering with arthritis.

Chronic inflammation may also involve pathological angiogenesis. Such disease states as ulcerative colitis and Crohn's disease show histological changes with the ingrowth of new blood  
15 vessels into the inflamed tissues. Bartonellosis, a bacterial infection found in South America, can result in a chronic stage that is characterized by proliferation of vascular endothelial cells. Another pathological role associated with angiogenesis is found in atherosclerosis. The plaques formed within the lumen of blood  
20 vessels have been shown to have angiogenic stimulatory activity.

One of the most frequent angiogenic diseases of  
25 childhood is the hemangioma. In most cases, the tumors are benign and regress without intervention. In more severe cases, the tumors progress to large cavernous and infiltrative forms and create clinical complications. Systemic forms of hemangiomas, the hemangiomatoses, have a high mortality rate. Therapy-resistant hemangiomas exist that cannot be treated with therapeutics  
30 currently in use.

Angiogenesis is also responsible for damage found in  
35 hereditary diseases such as Osler-Weber-Rendu disease, or hereditary hemorrhagic telangiectasia. This is an inherited disease characterized by multiple small angiomas, tumors of blood or lymph vessels. The angiomas are found in the skin and mucous membranes, often accompanied by epistaxis (nosebleeds) or gastrointestinal bleeding and sometimes with pulmonary or hepatic arteriovenous fistula.

Angiogenesis is prominent in solid tumor formation and metastasis. Angiogenic factors have been found associated with several solid tumors such as rhabdomyosarcomas, retinoblastoma, Ewing's sarcoma, neuroblastoma, and osteosarcoma. A tumor cannot expand without a blood supply to provide nutrients and remove cellular wastes. Tumors in which angiogenesis is important include solid tumors, and benign tumors such as acoustic neuroma, neurofibroma, trachoma and pyogenic granulomas. Prevention of angiogenesis could halt the growth of these tumors and the resultant damage to the animal due to the presence of the tumor.

It should be noted that angiogenesis has been associated with blood-borne tumors such as leukemias, any of various acute or chronic neoplastic diseases of the bone marrow in which unrestrained proliferation of white blood cells occurs, usually accompanied by anemia, impaired blood clotting, and enlargement of the lymph nodes, liver, and spleen. It is believed that angiogenesis plays a role in the abnormalities in the bone marrow that give rise to leukemia-like tumors.

Angiogenesis is important in two stages of tumor metastasis. The first stage where angiogenesis stimulation is important is in the vascularization of the tumor which allows tumor cells to enter the blood stream and to circulate throughout the body. After the tumor cells have left the primary site, and have settled into the secondary, metastasis site, angiogenesis must occur before the new tumor can grow and expand. Therefore, prevention of angiogenesis could lead to the prevention of metastasis of tumors and possibly contain the neoplastic growth at the primary site.

Knowledge of the role of angiogenesis in the maintenance and metastasis of tumors has led to a prognostic indicator for breast cancer. The amount of neovascularization found in the primary tumor was determined by counting the microvessel density in the area of the most intense neovascularization in invasive breast carcinoma. A high level of

microvessel density was found to correlate with tumor recurrence. Control of angiogenesis by therapeutic means could possibly lead to cessation of the recurrence of the tumors.

Angiogenesis is also involved in normal physiological processes such as reproduction and wound healing. Angiogenesis is an important step in ovulation and also in implantation of the blastula after fertilization. Prevention of angiogenesis could be used to induce amenorrhea, to block ovulation or to prevent implantation by the blastula.

In wound healing, excessive repair or fibroplasia can be a detrimental side effect of surgical procedures and may be caused or exacerbated by angiogenesis. Adhesions are a frequent complication of surgery and lead to problems such as small bowel obstruction.

Several kinds of compounds have been used to prevent angiogenesis. Taylor et al. have used protamine to inhibit angiogenesis, see Taylor et al., *Nature* 297:307 (1982). The toxicity of protamine limits its practical use as a therapeutic. Folkman et al. have disclosed the use of heparin and steroids to control angiogenesis. See Folkman et al., *Science* 221:719 (1983) and U.S. Patent Nos. 5,001,116 and 4,994,443. Steroids, such as tetrahydrocortisol, which lack gluco and mineral corticoid activity, have been found to be angiogenic inhibitors.

Other factors found endogenously in animals, such as a 4 kDa glycoprotein from bovine vitreous humor and a cartilage derived factor, have been used to inhibit angiogenesis. Cellular factors such as interferon inhibit angiogenesis. For example, interferon  $\alpha$  or human interferon  $\beta$  has been shown to inhibit tumor-induced angiogenesis in mouse dermis stimulated by human neoplastic cells. Interferon  $\beta$  is also a potent inhibitor of angiogenesis induced by allogeneic spleen cells. See Sidky et al., *Cancer Research* 47:5155-5161 (1987). Human recombinant  $\alpha$  interferon ( $\alpha/A$ ) was reported to be successfully used in the treatment of pulmonary hemangiomatosis, an angiogenesis-

induced disease. See White et al., *New England J. Med.* 320:1197-1200 (1989).

Other agents which have been used to inhibit angiogenesis include ascorbic acid ethers and related compounds. See Japanese Kokai Tokkyo Koho No. 58-131978. Sulfated polysaccharide DS 4152 also shows angiogenic inhibition. See Japanese Kokai Tokkyo Koho No. 63-119500. A fungal product, fumagillin, is a potent angiostatic agent *in vitro*. The compound is toxic *in vivo*, but a synthetic derivative, AGM 12470, has been used *in vivo* to treat collagen II arthritis. Fumagillin and O-substituted fumagillin derivatives are disclosed in EPO Publication Nos. 0325199A2 and 0357061A1.

PCT Application No. WO 92/14455 to Kaplan et al. is directed to a method for controlling abnormal concentration of TNF-a by administering thalidomide or thalidomide derivatives to a patient with toxic concentrations of TNF-a.

The above compounds are either topical or injectable therapeutics. Therefore, there are drawbacks to their use as a general angiogenic inhibitor and lack adequate potency. For example, in prevention of excessive wound healing, surgery on internal body organs involves incisions in various structures contained within the body cavities. These wounds are not accessible to local applications of angiogenic inhibitors. Local delivery systems also involve frequent dressings which are impracticable for internal wounds, and increase the risk of infection or damage to delicate granulation tissue for surface wounds.

Thus, a method and composition are needed that are capable of inhibiting angiogenesis and which are easily administered. A simple and efficacious method of treatment would be through the oral route. If an angiogenic inhibitor could be given by an oral route, the many kinds of diseases discussed above, and other angiogenic dependent pathologies, could be treated easily. The optimal dosage could be distributed in a form that the patient could self-administer.

### Summary of the Invention

In accordance with the present invention, compositions and methods are provided that are effective in inhibiting unwanted angiogenesis. These compositions are easily administered by different routes, including orally, and can be given in dosages that are safe and provide angiogenic inhibition at internal sites. The present invention provides a method of treating mammalian diseases mediated by undesired and uncontrolled angiogenesis by administering a composition comprising an anti-angiogenic compound in a dosage sufficient to inhibit angiogenesis.

The present invention also includes angiogenic inhibiting compounds that contain an epoxide group. These angiogenic inhibiting compounds can be administered to a human or animal alone or with epoxide hydrolase inhibiting compounds.

The present invention also includes compositions comprising an anti-angiogenesis compound and an antiinflammatory compound. The antiinflammatory compound can be either a steroidal or nonsteroidal antiinflammatory compound. Nonsteroidal antiinflammatory compounds, called NSAIDs, are preferred.

The present invention is especially useful for treating certain ocular neovascular diseases such as macular degeneration. The compounds which are contemplated as part of the present invention preferably can be given orally to the patient and thereby halt the progression of the disease. Other disease that can be treated using the present invention are diabetic retinopathy, neovascular glaucoma and retrolental fibroplasia.

Accordingly, it is an object of the present invention to provide a compound and method to inhibit unwanted angiogenesis in a human or animal.

It is yet another object of the present invention to provide a composition for inhibiting angiogenesis by oral administration of the composition.

It is another object of the present invention to provide a treatment for diseases mediated by angiogenesis.

It is yet another object of the present invention to provide a treatment for macular degeneration.

5 It is yet another object of the present invention to provide a treatment for all forms of proliferative vitreoretinopathy including those forms not associated with diabetes.

10 It is yet another object of the present invention to provide a treatment for solid tumors.

It is yet another object of the present invention to provide a method and composition for the treatment of blood-born tumors such as leukemia.

15 It is another object of the present invention to provide a method and composition for the treatment of hemangioma.

It is another object of the present invention to provide a method and composition for the treatment of retrolental fibroplasia.

20 It is another object of the present invention to provide a method and composition for the treatment of psoriasis.

It is another object of the present invention to provide a method and composition for the treatment of Kaposi's sarcoma.

25 It is another object of the present invention to provide a method and composition for the treatment of Crohn's diseases.

30 It is another object of the present invention to provide a method and composition for the treatment of diabetic retinopathy.

Another object of the present invention is to provide a method and composition comprising thalidomide and antiinflammatory drugs for the treatment of angiogenesis dependent diseases.

It is another object of the present invention is to provide a method and composition comprising thalidomide and steroidal antiinflammatory drugs for the treatment of angiogenesis dependent diseases.

5 It is yet another object of the present invention is to provide a method and composition comprising thalidomide and nonsteroidal, antiinflammatory drugs for the treatment of angiogenesis dependent diseases.

10 It is another object of the present invention is to provide a method and composition comprising angiogenesis inhibiting compounds and antiinflammatory drugs for the treatment of angiogenesis dependent diseases.

15 Yet another object of the present invention is to provide a method and composition comprising angiogenesis inhibiting compounds and steroidal antiinflammatory drugs for the treatment of angiogenesis dependent diseases.

20 Another object of the present invention is to provide a method and composition comprising angiogenesis inhibiting compounds and nonsteroidal antiinflammatory drugs for the treatment of angiogenesis dependent diseases.

It is an object of the present invention to provide a method and composition antiinflammatory drugs for the treatment of angiogenesis dependent diseases.

25 It is another object of the present invention to provide a method and composition comprising steroidal antiinflammatory drugs for the treatment of angiogenesis dependent diseases.

30 It is yet another object of the present invention to provide a method and composition comprising nonsteroidal antiinflammatory drugs for the treatment of angiogenesis dependent diseases.

Another object of the present invention is to provide a method and composition comprising thalidomide and antiinflammatory drugs for the treatment of cancer.

Yet another object of the present invention is to provide a method and composition comprising thalidomide and steroidal antiinflammatory drugs for the treatment of cancer.

Another object of the present invention is to provide a method and composition comprising thalidomide and nonsteroidal antiinflammatory drugs for the treatment of cancer.

It is an object of the present invention to provide a method and composition comprising antiinflammatory drugs for the treatment of cancer.

It is another object of the present invention to provide a method and composition comprising steroidal antiinflammatory drugs for the treatment of cancer.

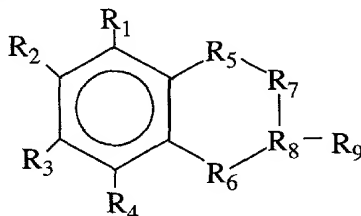
It is yet another object of the present invention to provide a method and composition comprising nonsteroidal antiinflammatory drugs for the treatment of cancer.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiments and the appended claims.

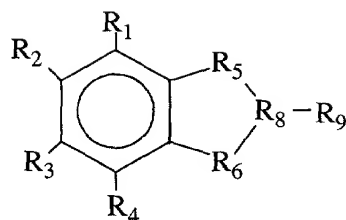
### Brief Description of the Figures

Figures 1 through 3 are a listing of representative compounds in the genus represented by the following general formulas:

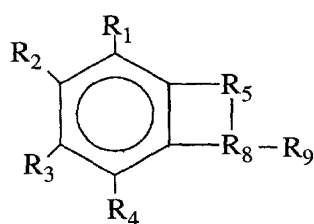
A)



B)

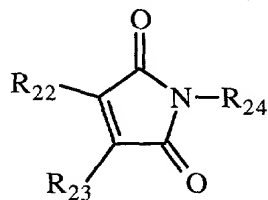


C)



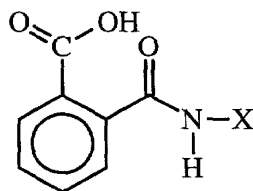
5

Figure 4 is a listing of representative compounds in the genus represented by the following general formula:



10

Figure 5 is a listing of representative compounds in the genus represented by the following general formula:



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Figure 6 shows the effect of thalidomide and EM12 on angiogenesis in a rabbit cornea model of angiogenesis.

Figure 7 shows the effect of thalidomide on the area of corneal vascularization in a rabbit cornea model of angiogenesis.

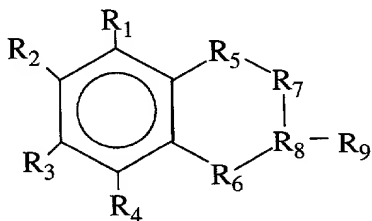
Figure 8 shows the effect of thalidomide, sulindac, or a combination of the two on the inhibition of tumor growth of V2-carcinoma in New Zealand White female rabbits.

## 5 Detailed Description

10 The present invention includes compositions and methods for the treatment of diseases that are mediated by angiogenesis. One embodiment of the present invention is the use of thalidomide or the metabolites of thalidomide as disclosed herein to inhibit unwanted angiogenesis. The present invention also includes compounds which cause dysmelia in the developing fetus and have anti-angiogenic activity. The present invention comprises a method of treating undesired angiogenesis in a human or animal comprising the steps of administering to the human or animal with the undesired angiogenesis a composition comprising an effective amount of a teratogenic compound that is anti-angiogenic.

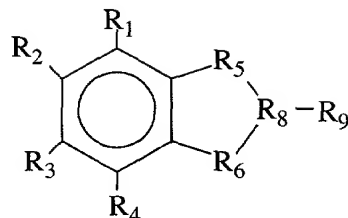
15 20 Compounds that can be used in accordance with the present invention include compounds included in the following general formulae. Examples of compounds that have anti-angiogenic properties having one of the following three formulae (A), (B), or (C):

A)

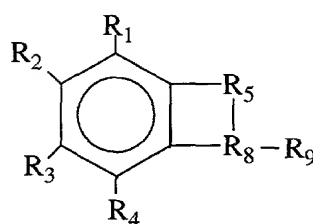


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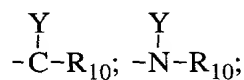
B)



C)

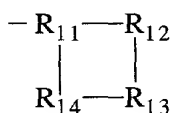


5 In the above formulae, A), B), and C), R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> can be selected from: -H; -OH; =O, straight and branched chain alkanes, alkenes, alkynes; cyclic alkanes, alkenes, and alkynes; combinations of cyclic and acyclic alkanes, alkenes, and alkynes; alcohol, aldehyde, ketone, carboxylic acids, esters, or ether moieties in combination with acyclic, cyclic, or combination acyclic/cyclic moieties; aza; amino; -XO<sub>n</sub> or -O-XO<sub>n</sub>, where X=N and n=2; X=S and n=2 or 3; or X=P and n=1-3; and halogens; R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, and R<sub>8</sub> are each independently selected from:

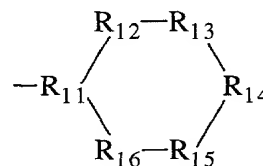


15 or -O- where Y is optional and is the same as defined above for R<sub>1</sub>; and R<sub>10</sub> is the same as defined above for R<sub>1</sub>, or when Y is absent, R<sub>10</sub> is =O; and R<sub>9</sub> is a moiety having formula D), E), F), G) or H):

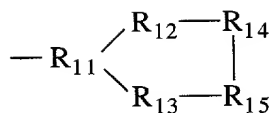
D)



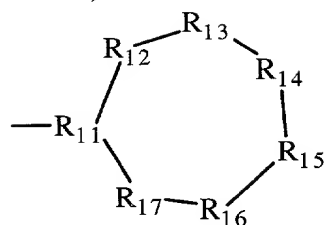
F)



E)



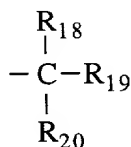
G)



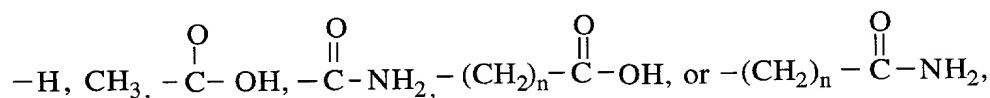
where each of R<sub>11</sub> - R<sub>17</sub> is independently the same as defined above for R<sub>5</sub>;

5

H)



where R<sub>18</sub>, R<sub>19</sub> and R<sub>20</sub> are, independently selected from



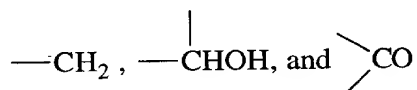
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and n=1 to 4.

Accordingly, another aspect of the present invention features inhibiting angiogenesis in a mammal by administering a therapeutic composition comprising one of the above-described compounds in a dosage sufficient to inhibit angiogenesis.

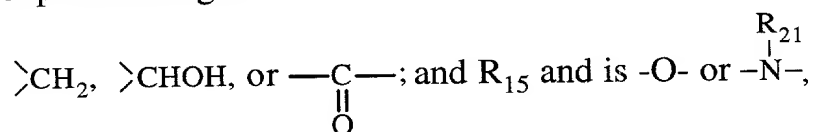
15

In preferred embodiments, the compound has formula B), where R<sub>5</sub> and R<sub>6</sub> are selected from the group consisting of:



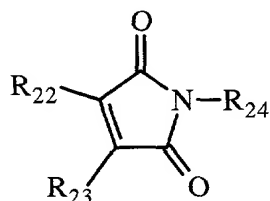
and R<sub>9</sub> has formula F) or H); and R<sub>14</sub> and R<sub>16</sub> are selected from the group consisting of:

20



where R<sub>21</sub> is -H, -CH<sub>3</sub>, or -OH. Specific preferred compounds according to this aspect of the present invention include thalidomide, its precursors, metabolites and analogs. Particular analogs include EM-12, N-phthaloyl-DL-glutamic acid (PGA) or N-phthaloyl-DL-glutamine anhydride. Examples of compounds that are members of this genus are listed in Figures 1 through 3. It is to be understood that the compounds included as part of the present invention are not to be limited to those compounds shown in Figures 1 through 3 and include all other compounds that are members of the genus described by the general formulas herein.

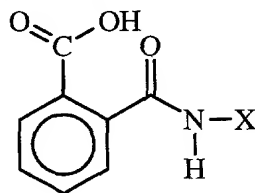
Compounds of the following formula that have anti-angiogenic properties:



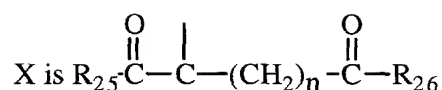
where R<sub>22</sub> and R<sub>23</sub> are (independently), -H, -F, -Cl, -Br, -I, -CH<sub>3</sub>, or -CH<sub>2</sub>-CH<sub>3</sub>; and R<sub>24</sub> is -H, -CH<sub>3</sub>, or -CH<sub>2</sub>-CH<sub>3</sub>.

The present invention also features inhibiting angiogenesis in a mammal by administering a compound according to the above formulae in a dosage sufficient to inhibit angiogenesis. Examples of specific compounds that are members of this genus are listed in Figure 4.

Angiogenesis inhibition hydrolysis products of thalidomide having the following general formula can be used in practicing the present invention:

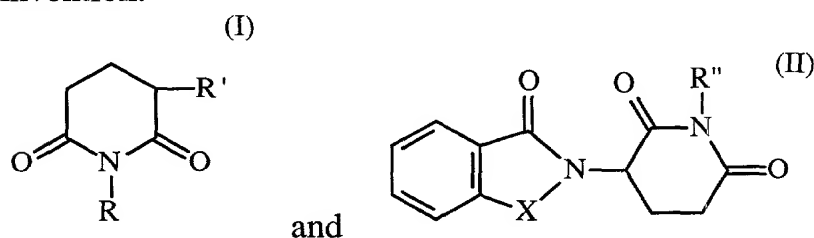


where X is R<sub>6</sub> as defined above, or

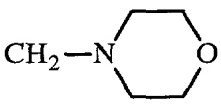


and R<sub>25</sub> and R<sub>26</sub> are, independently, -OH, -H, or NH<sub>2</sub>, and n=1 through 4. Examples of such compounds are shown in Figure 5.

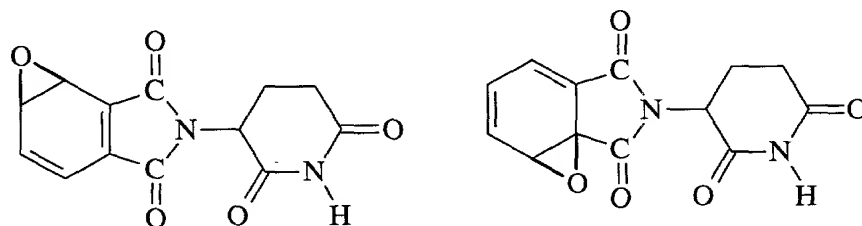
5                   Angiogenesis inhibition compounds having the following general formula can be used in practicing the present invention:



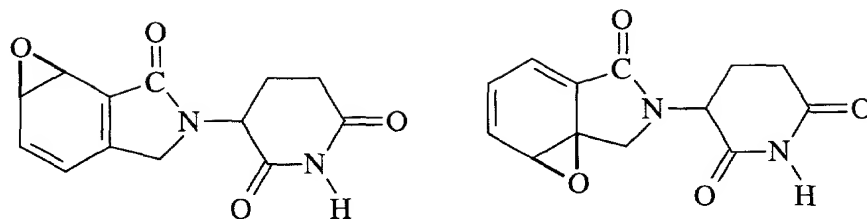
10                   wherein R is selected from the group consisting of hydrogen, alkyl radicals of 1 to 6 carbon atoms, the phenyl radical, and the benzyl radical; wherein R' is selected from the group consisting of the phthalimido radical and the succinimido radical; wherein X is CH<sub>2</sub> or C=O; and wherein R'' is H, -CH<sub>2</sub>CH<sub>3</sub>, -C<sub>6</sub>H<sub>5</sub>, -

15                   CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, -CH<sub>2</sub>CH=CH<sub>2</sub>, or ; and hydrolysis products of the compounds wherein R'' is H and the piperidino ring or both the piperidino and the imido ring are hydrolyzed.

20                   Another set of compounds that are considered part of the present invention are the epoxides of thalidomide, EM-12 and EM-138. Representative epoxide compounds are shown as follows:

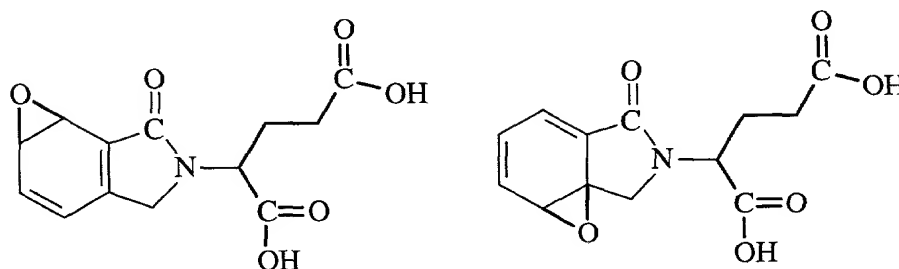


Epoxides of thalidomide



Epoxides of EM 12

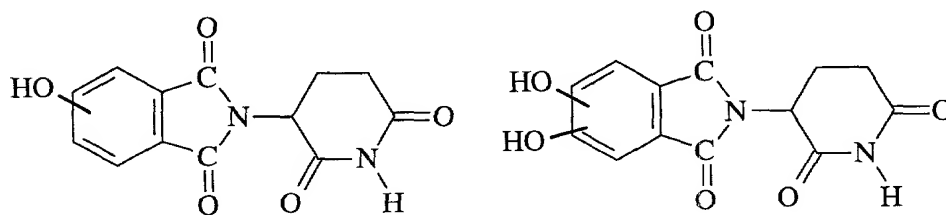
5



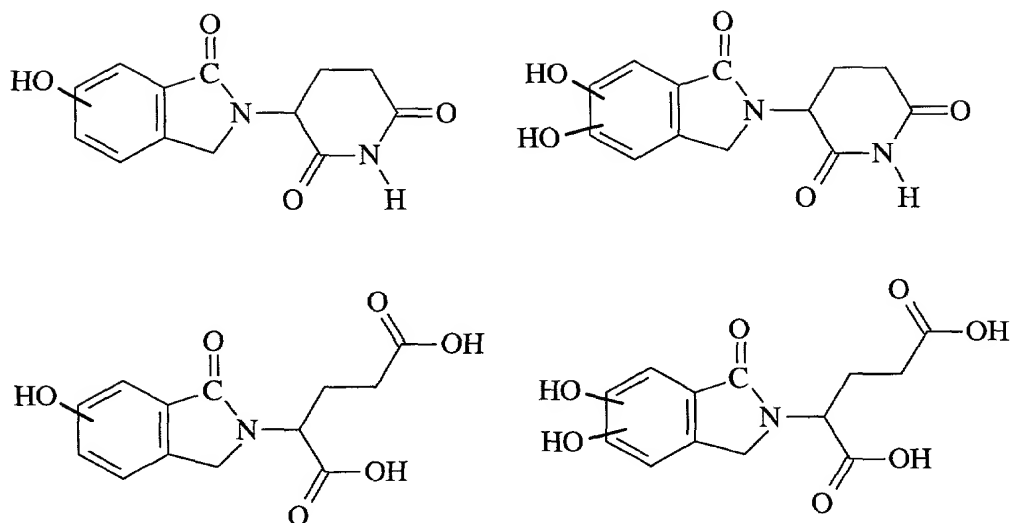
Epoxides of EM 138

It should be understood that the epoxide can be attached at the 6,1 site on the benzene ring, the 1,2 site, the 2,3 site 3,4 or the 4,5 site. All of these compounds are contemplated as part of the present invention.

The epoxides of the thalidomide, EM-12, and EM-138 can be hydrolyzed to the following compounds:



15



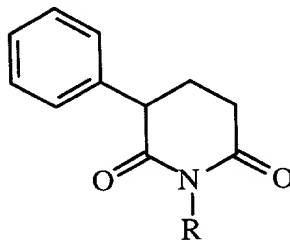
It is to be understood that the hydroxyl group can be on carbons 1, 2, 3, 4, 5 and 6 of the benzene ring. Also contemplated as part of the present invention are dihydroxyl compounds wherein the two hydroxyl groups are located bis to each other on carbons 1, 2, 3, 5 and 6 of the above compounds. The epoxides, the hydrolysis products of the epoxides, and the hydrolysis products of the thalidomide are all contemplated to be part of the present invention.

It is known that epoxides are hydrolized by a group of enzymes known as epoxide hydrolases. There is a class of compounds which are epoxide hydrolase inhibitors. Examples of these compounds are valpromide (2-propylpentanamide) and valproic acid (2-propylpentanoic acid). Because epoxides are important angiogenesis inhibitors, it is contemplated as part of the present invention, compositions comprising any of the angiogenesis inhibitors compounds recited herein in combination with epoxide hydrolase inhibitors. The epoxide hydrolase inhibitors can be administered to a human or animal together or sequentially. The epoxide group appears to be an important substituent common to several angiogenesis inhibitors. The use of epoxide hydrolase inhibitors to potentiate the activity of any angiogenesis inhibitor containing an epoxide is contemplated as

part of the present invention. For example, the epoxide hydrolase inhibitors can be administered with the following epoxide-containing anti-angiogenesis compounds: AGM 1470, Eponimycin, microbial metabolites of *Scolecobasidium arenarium* designated f/2015, fr/111142 and fr/18487. See Oikawa, *Biochem Biophys. Res. Comm.*, Vol. 81:1070 (1971) and Otsuka, *J. Microbial. Biotech.*, Vol 1:163 (1991).

It is contemplated as an embodiment of the present invention the use of the epoxide containing angiogenesis inhibitors with or without epoxide hydrolase inhibitors as a treatment for diseases mediated by elevated or toxic levels of TNF- $\alpha$ . TNF- $\alpha$  has been recognized as manifesting a dose dependent toxicity. If present at low levels for a long period of time, TNF- $\alpha$  can result in cachexia. Cachexia is a general weight loss and wasting occurring in the course of some chronic diseases such as cancer, opportunistic infections of AIDS, inflammatory diseases, parasitic diseases, tuberculosis, and high dose IL-2 therapy. The epoxide containing angiogenesis inhibitors, with or without epoxide hydrolase inhibitors, are also effective in treating diseases such as septic shock, leprosy and graft vs. host disease.

Other embodiments are within the present invention. For example, other dysmelia-causing compounds can be used according to the present invention, e.g. 4-methylphthalic acid, pyridoxine, vasopressin, acetazolamide, or a compound having the following formula (where R= H, -OH, or -CH<sub>3</sub>):



Other compounds which are teratogens, such as valproic acid (2-propylpentanoic acid), the retinoids, such as cis-retinoic acid, and rifampin may also be used in accordance with the invention.

In summary, the preferred compounds are thalidomide, as well as analogs, hydrolysis products, metabolites

and precursors of thalidomide that are teratogenic, and, more specifically, that cause dysmelia. However, it is to be understood that it is not necessary for a compound to have both teratogenic activity and angiogenesis inhibiting activity to be considered part of the present invention. Dysmelia-causing compounds can be identified by the general procedures of Helm, *Arzneimittelforschung*, 31(i/6):941-949 (1981), in which rabbit pups are examined after exposure to the compound in utero. The compounds can generally be purchased, e.g., from Andrulis Pharmaceuticals, Beltsville, MD, or synthesized according to known procedures. It is to be understood that the compounds of the present invention can exist as enantiomers and that the racemic mixture of enantiomers or the isolated enantiomers are all considered as within the scope of the present invention.

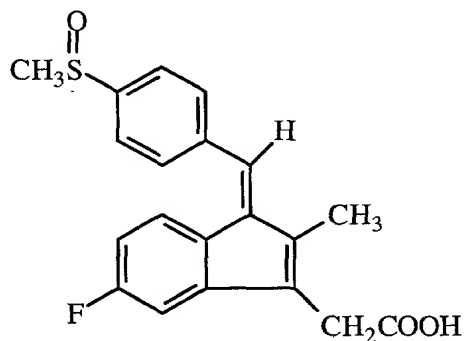
Many of the compounds that are contemplated as part of the present invention can be enriched in optically active enantiomers of the compounds specified above. Specifically, *Blaschke* has reported that the S enantiomers may be disproportionately responsible for the dysmelia-producing effect of these compounds. See, generally *Blaschke*, *Arzneimittelforschung* 29:1640-1642 (1979). The above described articles generally describe procedures to obtain optically active preparations of the compounds of interest. See, e.g. *Shealy et al.*, *Chem. Indus.* 1030 (1965); and *Casini et al.*, *Farmaco Ed. Sci.* 19:563 (1964).

In another embodiment, the invention also includes the inhibition of angiogenesis and the treatment of angiogenesis dependent diseases by administering antiinflammatory compounds, either alone or in combination with other angiogenesis inhibiting compounds, such as those described above. These antiinflammatory compounds may be either steroids or nonsteroidal antiinflammatory drugs (NSAIDs). Examples of steroids which may be used in the invention include, but are not limited to, cortisone, cortisol, corticosterone, hydrocortisone, hydrocortisol, prednisone, prednisolone, dexamethasone,

beclomethasone, betamethasone, mometasone, mometasone furoate, budesonide, triamcinolone acetonide, and fluticasone. Preferred steroids are prednisone, hydrocortisone, cortisol, dexamethasone, betamethasone, and beclomethasone. Especially preferred steroids are hydrocortisone, dexamethasone, and betamethasone.

Examples of NSAIDs which may be used in the invention include, but are not limited to, aspirin, acetaminophen, ibuprofen, esculetin, phenidone, quercetin, ketoprofen, nordihydroguaiaretic acid (NDGA), sulindac, sulindac sulfone, sulindac sulfide, indomethacin, NS-398 (a cyclooxygenase-2 inhibitor), cyclooxygenase-1 inhibitors, methylheptyl imidazole, furegrelate sodium, SKF525AHCL, thromboxane inhibitors, toradol, ecase, salsalate, diflunisal, mefenamic acid, naproxen, naproxen sodium, floctafenine, meclofenamate, phenylbutazone, oxyphenbutazone, diclofenac, etodolac, fenoprofen, flufenamic acid, flurbiprofen, piroprofen, tolmetin, apazone, fenbufen, nabumetone, oxaprozin, piroxicam, salicylate, and tenoxicam. Preferred NSAIDs are sulindac, sulindac sulfone, sulindac sulfide, indomethacin, NS-398, methylheptyl imidazole, furegrelate sodium, and SKF525AHCL. Especially preferred NSAIDs are indomethacin and sulindac.

Sulindac, which includes (Z)-5-Fluoro-2-methyl-1-[[4-(methyl-sulfinyl)phenyl] methylene]-1H-indene-3-acetic acid, or cis-5-fluoro-2-methyl-1-[p-(methylsulfinyl)benzylidene]indene-3-acetic acid, has the following structure:



Such compounds can be used to treat angiogenesis dependent diseases. Such compounds can be used alone or in combination with other angiogenesis inhibiting compounds to treat angiogenesis dependent diseases, such as cancer.

5           The compounds described above can be provided as pharmaceutically acceptable formulations using formulation methods known to those of ordinary skill in the art. These formulations can be administered by standard routes. In general, the combinations may be administered by the topical, transdermal, 10 oral, rectal or parenteral (e.g., intravenous, subcutaneous or intramuscular) route. In addition, the combinations may be incorporated into biodegradable polymers allowing for sustained release of the compound, the polymers being implanted in the vicinity of where drug delivery is desired, for example, at the site of a tumor. The biodegradable polymers and their use are 15 described, for example, in detail in Brem et al., *J. Neurosurg.* 74:441-446 (1991).

20           The dosage of the compound will depend on the condition being treated, the particular compound, and other clinical factors such as weight and condition of the human or animal and the route of administration of the compound. It is to be understood that the present invention has application for both human and veterinary use. For oral administration to humans, a dosage of between approximately 0.1 to 300 mg/kg/day, preferably between approximately 0.5 and 50 mg/kg/day, and 25 most preferably between approximately 1 to 10 mg/kg/day, is generally sufficient.

30           The formulations include those suitable for oral, rectal, ophthalmic, (including intravitreal or intracameral) nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intratracheal, and epidural) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by conventional pharmaceutical techniques. Such 35 techniques include the step of bringing into association the active

ingredient and the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil emulsion and as a bolus, etc.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface active or dispersing agent. Molded tablets may be made by molding, in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be optionally coated or scored and may be formulated so as to provide a slow or controlled release of the active ingredient therein.

Formulations suitable for topical administration in the mouth include lozenges comprising the ingredients in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the ingredient to be administered in a suitable liquid carrier.

Formulations suitable for topical administration to the skin may be presented as ointments, creams, gels and pastes comprising the ingredient to be administered in a pharmaceutical acceptable carrier. A preferred topical delivery system is a transdermal patch containing the ingredient to be administered.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

5 Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of 20 to 500 microns which is administered in the manner in which snuff is administered, i.e., by rapid inhalation through the nasal passage from a container of the powder held close up to the nose.  
10 Suitable formulations, wherein the carrier is a liquid, for administration, as for example, a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or  
15 spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which  
20 render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be  
25 stored in freeze-dried (lyophilized) conditions requiring only the addition of the sterile liquid carrier, for example, water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

30 Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, as herein above recited, or an appropriate fraction thereof, of the administered ingredient.

It should be understood that in addition to the  
35 ingredients, particularly mentioned above, the formulations of the

present invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include flavoring agents.

5 Diseases associated with corneal neovascularization that can be treated according to the present invention include but are not limited to, diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma and  
10 retrolental fibroplasia, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogren's syndrome, acne rosacea, phlyctenulosis, syphilis, *Mycobacteria* infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, *Herpes simplex* infections, *Herpes zoster* infections, protozoan  
15 infections, Kaposi's sarcoma, Mooren's ulcer, Terrien's marginal degeneration, marginal keratolysis, trauma, rheumatoid arthritis, systemic lupus, polyarteritis, Wegener's sarcoidosis, scleritis, Stevens-Johnson disease, radial keratotomy, pemphigoid and corneal graft rejection.

20 Diseases associated with retinal/choroidal neovascularization that can be treated according to the present invention include, but are not limited to, diabetic retinopathy, macular degeneration, sickle cell anemia, sarcoid, syphilis, pseudoxanthoma elasticum, Paget's disease, vein occlusion, artery  
25 occlusion, carotid obstructive disease, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus erythematosus, retinopathy of prematurity, Eales' disease, Behcet's disease, infections causing a retinitis or choroiditis, presumed ocular histoplasmosis, Best's disease, myopia, optic pits, Stargardt's disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser  
30 complications. Other diseases include, but are not limited to, diseases associated with rubeosis (neovascularization of the angle) and diseases caused by the abnormal proliferation of fibrovascular

or fibrous tissue including all forms of proliferative vitreoretinopathy, whether or not associated with diabetes.

Diseases associated with chronic inflammation can be treated by the compositions and methods of the present invention. Diseases with symptoms of chronic inflammation include inflammatory bowel diseases such as Crohn's disease and ulcerative colitis, psoriasis, sarcoidosis and rheumatoid arthritis. Angiogenesis is a key element that these chronic inflammatory diseases have in common. The chronic inflammation depends on continuous formation of capillary sprouts to maintain an influx of inflammatory cells. The influx and presence of the inflammatory cells produce granulomas and, thus, maintain the chronic inflammatory state. Inhibition of angiogenesis by the compositions and methods of the present invention would prevent the formation of the granulomas and alleviate the disease.

The compositions and methods of the present invention can be used to treat patients with inflammatory bowel diseases such as Crohn's disease and ulcerative colitis. Both Crohn's disease and ulcerative colitis are characterized by chronic inflammation and angiogenesis at various sites in the gastrointestinal tract. Crohn's disease is characterized by chronic granulomatous inflammation throughout the gastrointestinal tract consisting of new capillary sprouts surrounded by a cylinder of inflammatory cells. Prevention of angiogenesis by the compositions and methods of the present invention inhibits the formation of the sprouts and prevents the formation of granulomas.

Crohn's disease occurs as a chronic transmural inflammatory disease that most commonly affects the distal ileum and colon but may also occur in any part of the gastrointestinal tract from the mouth to the anus and perianal area. Patients with Crohn's disease generally have chronic diarrhea associated with abdominal pain, fever, anorexia, weight loss and abdominal swelling. Ulcerative colitis is also a chronic, nonspecific,

inflammatory and ulcerative disease arising in the colonic mucosa and is characterized by the presence of bloody diarrhea.

5 The inflammatory bowel diseases also show extraintestinal manifestations such as skin lesions. Such lesions are characterized by inflammation and angiogenesis and can occur at many sites other than the gastrointestinal tract. The compositions and methods of the present invention are also capable of treating these lesions by preventing the angiogenesis, thus, reducing the influx of inflammatory cells and the lesion formation.

10 Sarcoidosis is another chronic inflammatory disease that is characterized as a multisystem granulomatous disorder. The granulomas of this disease may form anywhere in the body, and, thus, the symptoms depend on the site of the granulomas and whether the disease active. The granulomas are created by the angiogenic capillary sprouts providing a constant supply of inflammatory cells.

15 The compositions and methods of the present invention can also treat the chronic inflammatory conditions associated with psoriasis. Psoriasis, a skin disease, is another chronic and recurrent disease that is characterized by papules and plaques of various sizes. Prevention of the formation of the new blood vessels necessary to maintain the characteristic lesions leads to relief from the symptoms.

20 Another disease which can be treated according to the present invention is rheumatoid arthritis. Rheumatoid arthritis is a chronic inflammatory disease characterized by nonspecific inflammation of the peripheral joints. It is believed that the blood vessels in the synovial lining of the joints undergo angiogenesis. In addition to forming new vascular networks, the endothelial cells release factors and reactive oxygen species that lead to pannus growth and cartilage destruction. The factors involved in angiogenesis may actively contribute to, and help maintain, the chronically inflamed state of rheumatoid arthritis.

Other diseases that can be treated according to the present invention are hemangiomas, Osler-Weber-Rendu disease, or hereditary hemorrhagic telangiectasia, solid or blood borne tumors and acquired immune deficiency syndrome.

5           The compositions and methods of the present invention include the use of angiogenesis inhibiting compounds and antiinflammatory compounds, such as steroids or nonsteroidal antiinflammatory drugs (NSAIDs). The compositions and  
10           methods of the present invention include the combination of angiogenesis inhibiting compounds, such as thalidomide or thalidomide-like analogs or AMG-1470, EM-12 or EM-138 with antiinflammatory compounds, such as steroids or nonsteroidal antiinflammatory drugs (NSAIDs). The compositions and  
15           methods of the present invention also include the use antiinflammatory compounds alone. These compositions can be used to treat angiogenesis dependent diseases.

          For example, it has been found that indomethacin (5 mg/kg) inhibits bFGF induced angiogenesis by 59% and VEGF induced angiogenesis by 61%. Similarly, sulindac (25 mg/kg) has  
20           been found to inhibit bFGF induced angiogenesis by 50% and VEGF induced angiogenesis by 55%. Sulindac is metabolized in vivo to two metabolites: sulindac sulfide and sulindac sulfone. Sulindac sulfide actively inhibits prostaglandin synthesis, while sulindac sulfone does not; however, both were found to be  
25           inhibitors of angiogenesis. Sulindac sulfide was found to inhibit bFGF induced neovascularization by 34%, and sulindac sulfone exhibited 31% inhibition.

          Other NSAIDs have also been found to inhibit angiogenesis associated neovascularization. For example,  
30           carbomethylheptyl imidazole and furegrelate sodium, both thromboxane inhibitors, inhibit bFGF induced neovascularization 32% and 22%, respectively. Another thromboxane inhibitor, SKF525AHCL, which promotes prostaglandin production, was found to inhibit bFGF induced neovascularization by 25%. The

specific cyclooxygenase-2 inhibitor NS-398 also inhibited bFGF induced neovascularization by 25%.

When sulindac is combined with thalidomide, there is an additive effect in the inhibition of angiogenesis. When sulindac is combined with other angiogenesis inhibiting compounds, such as AMG 1470, EM-12 or EM-138, there is an additive effect in the inhibition of angiogenesis. Angiogenesis in the eye, in the assay described in Example 2, is produced by the presence of bFGF (basic fibroblastic growth factor) and VEGF (vascular endothelial cell growth factor). Inhibition of such angiogenesis was shown with the NSAIDs, steroids, thalidomide, or the combination of thalidomide and an NSAID, sulindac.

5

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| AGENT                  | DOSE  | bFGF <sup>1</sup> | VEGF <sup>2</sup> |
|------------------------|---|-------------------|-------------------|
| Aspirin                | 100 mg/kg                                   | 8                 | -                 |
| Acetaminophen          | 100 mg/kg                                   | 0                 | 0                 |
| Ibuprofen              | 20 mg/kg                                    | 7                 | 11                |
| Hydrocortisone         | 20 mg/kg                                    | 50                | 40                |
| Sulindac               | 25 mg/kg                                    | 52                | 54                |
| Thalidomide            | 200 mg/kg                                   | 42                | 44                |
| Thalidomide + Sulindac | 200 mg/kg (thal) +<br>25 mg/kg (sulindac)   | 65                | 74                |
| AGM 1470               | 30 mg/kg qod                                | 48                |                   |
| AGM 1470 + Sulindac    | 30 mg/kg qod (AGM)<br>+ 25 mg/kg (sulindac) | 70                |                   |

<sup>1</sup>% Inhibition of bFGF induced angiogenesis

<sup>2</sup>% Inhibition of VEGF induced angiogenesis

5                   The inhibition of VEGF by 74% demonstrates the additive effect when thalidomide and sulindac are combined. The data for hydrocortisone, sulindac, thalidomide and thalidomide + sulindac differ significantly from the controls ( $p < 0.0001$ ). Studies with the composition comprising thalidomide and sulindac on V2 carcinoma in rabbits has demonstrated a T/C (treated to control ratio) of 0.32 after 18 days of oral treatment with thalidomide 200 mg/kg combined with 25 mg/kg sulindac.

10                   An additive effect is also seen in the combination of nonsteroidal antiinflammatory drugs and other angiogenesis inhibiting compounds such as the combination of sulindac and AMG-1470. AGM-1470 is a known angiogenesis inhibiting compound as shown in Brem et al. Minimal drug resistance occurs after prolonged antiangiogenic therapy with AGM-1470, *Surgical Forum* 45 (0), 1994, pp 674-677, herein incorporated by reference. As shown in the above chart, sulindac plus AGM-1470 shows a greater inhibitory effect upon angiogenesis than does either compound alone.

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implantation by gastric lavage with either drug suspended in 0.5% carboxymethyl cellulose or 0.5% carboxymethyl cellulose alone. Thalidomide was purchased from Andrulus Pharmaceutical (Maryland) and the EM-12 and Supidimide were kindly provided by Grunenthal GMBH (Germany). The animals were examined with a slit lamp every other day in a masked manner by the same corneal specialist. The area of corneal neovascularization was determined by measuring with a reticule the vessel length (L) from the limbus and the number of clock hours (C) of limbus involved. A formula was used to determine the area of a circular band segment:  $C/12 * 3.1416 [r^2 - (r-L)^2]$  where  $r=6$  mm the measured radius of the rabbit cornea. Various mathematical models were utilized to determine the amount of vascularized cornea, and this formula was found to provide the most accurate approximation of the area of the band of neovascularization that grows towards the pellet.

It is important to note that the rabbit cornea assay is preferable because it will generally recognize compounds that are inactive *per se* but are metabolized to yield active compounds. Thalidomide related compounds, as shown below in Example III, are known to be teratogens and are candidates for use in the present invention.

### Example III

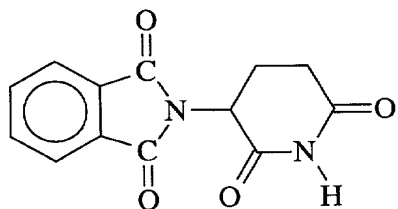
*Inhibition of bFGF induced corneal neovascularization by thalidomide and related analog expressed as percent of median control on day 8*

Pellets containing bFGF and sucralfate were implanted into micropockets of both corneas of rabbits according to Example II. Vessel ingrowth into clear cornea from the limbus was first noted on day 2 and treatments (200 mg/kg orally) were begun on this day. The area of corneal neovascularization was measured from day 4 through day 12. Day 8 measurements were used for comparison between groups. No regression of vessels and near maximal neovascularization was seen at this time point.

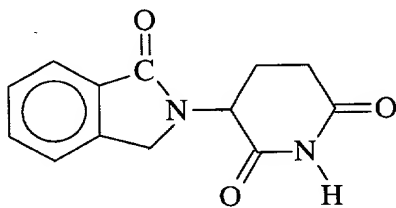
Statistical analysis was performed with ANOVA with ranked data to account for interexperimental variation and to guard against a non-normal distribution of data (i.e. outliers) by utilizing a nonparametric method.

5

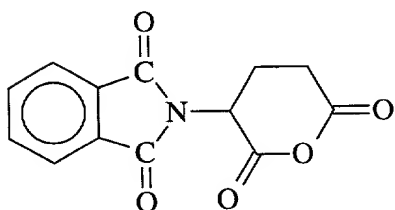
The compounds tested were as follows:



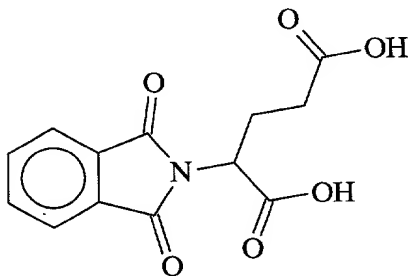
thalidomide



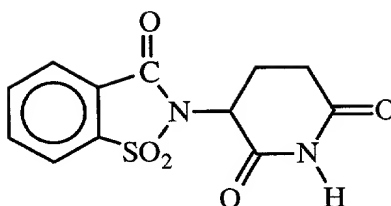
EM-12



phthaloyl glutamic anhydride (PGA)



phthaloyl glutamic acid (PG Acid)



supidimide.

Treatment with a dose of (200 mg/kg) of thalidomide resulted in an inhibition of the area of vascularized cornea that ranged from 30-51% in three experiments with a median inhibition of 36% (Figure 6) (n=30 eyes, p=0.0001, 2 way ANOVA with ranked data). The inhibition of angiogenesis by thalidomide was seen after only two doses (Figure 7). The rabbits did not demonstrate obvious sedation and there were no signs of toxicity or weight loss. The teratogenic analog EM-12, which shares the other properties of thalidomide was also inhibitory, with a median inhibition of 42% (n=10 eyes, p=0.002, 1-way ANOVA with ranked data). Supidimide, a nonteratogenic analog of thalidomide that retains the sedative properties of thalidomide, exhibited no activity (area 107% of control, n=10 eyes, not statistically different from control). Other analogs, PGA and PG acid displayed weaker inhibitory effects than thalidomide (data not shown). The density of vessel ingrowth in thalidomide-treated animals was also markedly reduced.

#### Example IV

##### *EM-12 in rabbit cornea assay*

EM-12 was tested in the rabbit cornea assay described in Example II at 100 mg/kg/day and showed 21% inhibition, and at 200 mg/kg/day the assay showed 43% inhibition.

#### Example V

##### *Phthaloyl glutamic acid in CAM*

Phthaloyl glutamic acid was tested in the above described CAM assay and exhibit an avascular zone with a mild scar.

Example VI

*Phthaloyl glutamic acid in rabbit cornea assay*

5 Phthaloyl glutamic acid described above at 200 mg/kg and exhibited 29% inhibition of angiogenesis.

Example VII

*Phthaloyl glutamic anhydride in CAM assay*

10 Phthaloyl glutamic anhydride was test in the CAM assay described above and exhibited an avascular zone.

Example VIII

*Treatment of Crohn's Disease*

15 A 32 year old female patient with Crohn's disease was treated using the methods of the present invention. The patient exhibited the characteristic symptoms of Crohn's disease, i.e., severe gastrointestinal involvement (including diarrhea and cramping) and a large skin lesion on the lower leg. Thalidomide was orally administered to her at a dosage of 100 mg twice a day.  
20 After treatment was continued for one week, the gastrointestinal symptoms, including the diarrhea and cramping, were lessened and the skin lesion resolved.

Example IX

25 Corneal Micropocket Assay

Six to eight week old C57B16 male mice were obtained from Jackson Laboratories, MA. The mice were anesthetized, and 0.4 x 0.4 mm pockets were made in the stroma of the mouse cornea adjacent to the limbus. Pellets containing 80  
30 ng of either bFGF or VEGF were implanted in the pellets. The pellets containing bFGF were implanted 1.0-1.2 mm from the limbal vessels, while the pellets containing VEGF were implanted 0.5-0.7 mm from the limbal vessels. Erythromycin was then topically applied.

The mice were then treated with varying doses of antiinflammatory drugs as shown in the table below. The vascular response to the pellets was measured by maximal vessel length and number of clock hours of neovascularization 5 days after implantation of the bFGF pellets and 6 days after implantation of the VEGF pellets. The area of corneal neovascularization was calculated using the following formula which best approximated the area of neovascularization: Area (mm<sup>2</sup>) = [ $\pi$  x clock hours x length (mm) x 0.2(mm)]. The results are shown in the following table.

| AGENT         | DOSE         | bFGF <sup>1</sup> | VEGF <sup>2</sup> | n     | p-value   |
|---------------|--------------|-------------------|-------------------|-------|-----------|
| Acetaminophen | 100 mg/kg    | 0                 | -                 | 8     | ns        |
| Aspirin       | 10-160 mg/kg | 0-11              | -                 | 8     | ns        |
| NDGA          | 25 mg/kg     | 30                | -                 | 8     | ns        |
| Esculetin     | 200 mg/kg    | 15                | -                 | 8     | .02       |
| Phenidone     | 100 mg/kg    | 17                | -                 | 8     | <.01      |
| Quercetin     | 300 mg/kg    | 18                | -                 | 8     | <.01      |
| Ibuprofen     | 25 mg/kg     | 6                 | 8                 | 23/8  | ns/ns     |
| Ketoprofen    | 80 mg/kg     | 30                | 41                | 8/8   | <.01      |
| Indomethacin  | 5 mg/kg      | 59                | 61                | 15/21 | <.01/<.01 |
| Sulindac      | 25 mg/kg     | 50                | 55                | 15/15 | <.01/<.01 |

<sup>1</sup>% Inhibition of bFGF induced angiogenesis

<sup>2</sup>% Inhibition of VEGF induced angiogenesis

Inhibitory effect is expressed in percentage representing the area of corneal neovascularization by either bFGF or VEGF compared to controls (n= 8/experiment) of the experiments in which that particular drug was tested. N= the number of eyes that were tested and drugs were given once daily either sc, ip or oral as described.

#### Example X

Six to eight week old C57B16 male mice were obtained from Jackson Laboratories, MA. The mice were

anesthetized, and 0.4 x 0.4 mm pockets were made in the stroma of the mouse cornea adjacent to the limbus. Pellets containing 80 ng of either bFGF or VEGF were implanted. The pellets containing bFGF were implanted 1.0-1.2 mm from the limbal vessels, while the pellets containing VEGF were implanted 0.5-0.7 mm from the limbal vessels. Erythromycin was then topically applied.

The mice were then treated with thalidomide, indomethacin, sulindac, or combinations of thalidomide with either indomethacin or sulindac. The vascular response to the pellets was measured by maximal vessel length and number of clock hours of neovascularization 5 days after implantation of the bFGF pellets and 6 days after implantation of the VEGF pellets. The area of corneal neovascularization was calculated using the following formula which best approximates the area of neovascularization:  $\text{Area (mm}^2\text{)} = [\pi \times \text{clock hours} \times \text{length (mm)} \times 0.2(\text{mm})]$ . The results are shown in the following table.

| AGENT                         | DOSE                    | bFGF <sup>1</sup> | VEGF <sup>2</sup> | n     |
|-------------------------------|-------------------------|-------------------|-------------------|-------|
| Thalidomide                   | 200 mg/kg               | 41                | 40                | 31/39 |
| Indomethacin                  | 5 mg/kg                 | 59                | 61                | 15/21 |
| Sulindac                      | 25 mg/kg                | 50                | 55                | 15/15 |
| Thalidomide +<br>Indomethacin | 200 mg/kg<br>+ 5 mg/kg  | 67                | 61                | 15/21 |
| Thalidomide +<br>Sulindac     | 200 mg/kg<br>+ 25 mg/kg | 63 <sup>3</sup>   | 74 <sup>3</sup>   | 15/16 |

<sup>1</sup>% Inhibition of bFGF induced angiogenesis

<sup>2</sup>% Inhibition of VEGF induced angiogenesis

<sup>3</sup>Inhibitory effect is significantly different from either agent alone

(p<.01, tested by ANOVA)

Inhibitory effect is expressed in percentage representing the area of corneal neovascularization by either bFGF or VEGF compared to controls (n= 8/experiment) of the experiments in which that particular drug was tested. N= the number of eyes that were

tested and drugs were given once daily either sc, ip or oral as described.

#### Example XI

Six to eight week old C57B16 male mice, obtained from Jackson Laboratories, MA, were anesthetized, and 0.4 x 0.4 mm pockets were made in the stroma of the mouse cornea adjacent to the limbus. Pellets containing 80 ng of bFGF were implanted 1.0-1.2 mm from the limbal vessels. Erythromycin was then topically applied.

The mice were then treated with sulindac, or one of the sulindac derivatives, sulindac sulfone or sulindac sulfide. The vascular response to the pellets was measured by maximal vessel length and number of clock hours of neovascularization 5 days after implantation of the bFGF pellets. The area of corneal neovascularization was calculated using the following formula which best approximates the area of neovascularization:  $\text{Area (mm}^2\text{)} = [\pi \times \text{clock hours} \times \text{length (mm)} \times 0.2(\text{mm})]$ . The results are shown in the following table.

| AGENT            | DOSE     | % inhibition | n  |
|------------------|----------|--------------|----|
| Sulindac         | 25 mg/kg | 50           | 15 |
| Sulindac sulfide | 25 mg/kg | 31           | 8  |
|                  | 50 mg/kg | 34           | 8  |
| Sulindac sulfone | 25 mg/kg | 31           | 16 |

Inhibitory effect is expressed in percentage representing the area of corneal neovascularization by bFGF compared to controls (n= 8/experiment. N= the number of eyes that were tested and drugs were given once daily either sc, ip or oral as described.

## Example XII

### Tumor Assay

New Zealand White female rabbits, weighing approximately 1.5 kg, obtained from Charles River, MA, were used for propagating the V2-carcinoma. This tumor originates from a Shope virus-induced papilloma. Small 0.5 x 0.5 cm pieces were implanted intramuscularly in the right thigh. Treatment with 200 mg/kg/day thalidomide (n = 14), 60 mg/kg/day sulindac (n = 5), or a combination of thalidomide and sulindac (n = 10) was started at day 10 after tumor implantation, when the mean volume of the tumor was 6 cm<sup>3</sup>. The control animals (n = 13) were treated with methylcellulose. The rabbits were sacrificed 17 days after the start of treatment when mean volume of the control tumors was 100 cm<sup>3</sup>. The results of this experiment (Figure 8) show that the combination of thalidomide and sulindac is more effective in reducing the size of V2-carcinoma tumors than either thalidomide or sulindac alone. The combination of thalidomide and sulindac inhibited tumor growth by 75% and was significantly different (p<0.05) from either agent alone or the control group. Oral treatment with sulindac or thalidomide inhibited tumor growth by, respectively, 35% (n = 5, p<0.01) and 55% (n = 14, p<0.01). The data were collected in 3 separate experiments and each bar represents the standard error of the mean.

It should be understood, of course, that the foregoing relates only to preferred embodiments of the present invention and that numerous modifications or alterations may be made therein without departing from the spirit and the scope of the invention as set forth in the appended claims.

We claim

1. An angiogenesis inhibitory composition comprising an angiogenesis inhibiting compound and an antiinflammatory drug.

5

2. The angiogenesis inhibitory composition of Claim 1 wherein the antiinflammatory drug is a steroid.

3. The angiogenesis inhibitory composition of Claim 2 wherein the steroid is selected from the group consisting of cortisol, corticosterone, hydrocortisone, hydrocortisol, cortisone, prednisone, prednisolone, dexamethasone, beclomethasone, betamethasone, mometasone, mometasone furoate, budesonide, triamcinolone acetonide, and fluticasone.

15

4. The angiogenesis inhibitory composition of Claim 1 wherein the antiinflammatory drug is a nonsteroidal, antiinflammatory drug (NSAID).

20

5. The angiogenesis inhibitory composition of Claim 4 wherein the NSAID is selected from group consisting of aspirin, acetaminophen, ibuprofen, esculetin, phenidone, quercetin, ketoprofen, nordihydroguaiaretic acid (NDGA), sulindac, sulindac sulfone, sulindac sulfide, indomethacin, NS-398 (a cyclooxygenase-2 inhibitor), cyclooxygenase-1 inhibitors, methylheptyl imidazole, furegrelate sodium, SKF525AHCL, thromboxane inhibitors, toradol, ecasa, salsalate, diflunisal, mefenamic acid, naproxen, naproxen sodium, floctafenine, meclofenamate, phenylbutazone, oxyphenbutazone, diclofenac, etodolac, fenoprofen, flufenamic acid, flurbiprofen, pirprofen, tolmetin, apazone, fenbufen, nabumetone, oxaprozin, piroxicam, salicylate, and tenoxicam.

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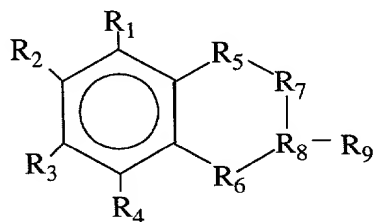
6. The angiogenesis inhibitory composition of Claim 5 wherein the NSAID is selected from indomethacin and sulindac.

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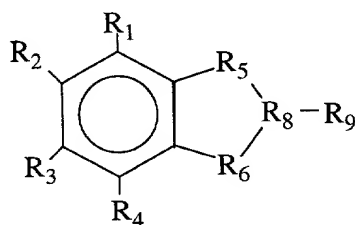
7. The angiogenesis inhibitory composition of Claim 1 wherein the angiogenesis inhibiting compound is selected from the group consisting of

5 (1) a compound selected from the formula

A)



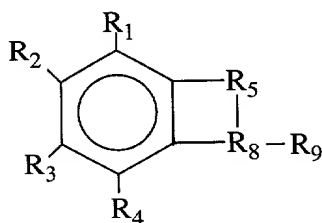
B)



10

or

C)



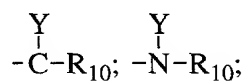
15 wherein

R<sub>1</sub> - R<sub>4</sub> are each independently selected from H; OH; =O; straight or branched chain alkanes, alkenes, and alkynes; cyclic alkanes, alkenes, and alkynes; combinations of cyclic and acyclic alkanes, alkenes, and alkynes; alcohol, aldehyde, ketone, carboxylic acid, ester, or other moieties in combination with acyclic, cyclic, or combination acyclic/cyclic moieties; aza; amino; -XO<sub>n</sub> or -O-

20

$\text{XO}_n$ , where  $\text{X}=\text{N}$  and  $n=2$ ,  $\text{X}=\text{S}$  and  $n=2$  or  $3$ , or  $\text{X}=\text{P}$  and  $n=1-3$ ; and halogens;

$\text{R}_5 - \text{R}_8$  are each independently selected from



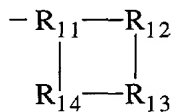
5

or  $-\text{O}-$ , where  $\text{Y}$  is absent and  $\text{R}_{10}$  is  $=\text{O}$  or  $\text{Y}$  and  $\text{R}_{10}$  are each independently the same as  $\text{R}_1$ ;

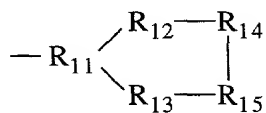
and  $\text{R}_9$  is a moiety selected from the group consisting of

10

D)

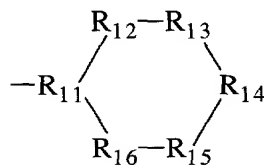


E)

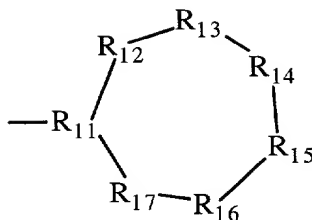


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F)

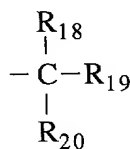


G)



5

and H)



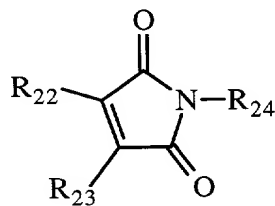
10

wherein each of R<sub>11</sub> - R<sub>17</sub> is independently the same as R<sub>5</sub>, and wherein R<sub>18</sub>, R<sub>19</sub> and R<sub>20</sub> are each independently selected from

H, CH<sub>3</sub>,  $\text{-}\overset{\text{O}}{\parallel}\text{C}\text{-OH}$ ,  $\text{-}\overset{\text{O}}{\parallel}\text{C}\text{-NH}_2$ ,  $\text{-(CH}_2\text{)}_n\text{-}\overset{\text{O}}{\parallel}\text{C}\text{-OH}$ , and  $\text{-(CH}_2\text{)}_n\text{-}\overset{\text{O}}{\parallel}\text{C}\text{-NH}_2$ ,  
and n=1 through 4;

15

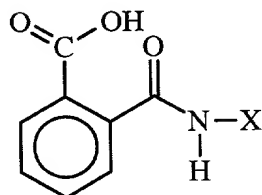
(2) a compound selected from the formula



20

where R<sub>22</sub> and R<sub>23</sub> are each independently H, F, Cl, Br, I, CH<sub>3</sub>, or  
-CH<sub>2</sub>-CH<sub>3</sub>;  
and R<sub>24</sub> is H, CH<sub>3</sub>, or -CH<sub>2</sub>-CH<sub>3</sub>;  
and

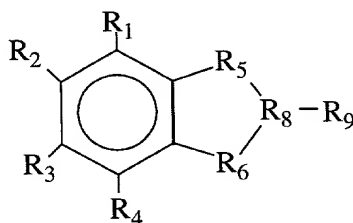
(3) a compound selected from the formula



5 where X is R<sub>6</sub> as defined in (1) above.

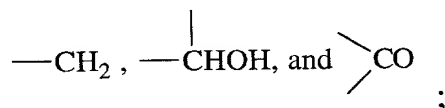
8. The angiogenesis inhibitory composition of Claim 7 wherein the angiogenesis inhibiting compound has the formula

B)



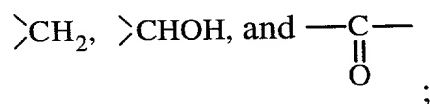
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wherein R<sub>1</sub>-R<sub>4</sub> are as defined in Claim 8;  
R<sub>5</sub> and R<sub>6</sub> are independently selected from the group consisting of



15

and R<sub>9</sub> is selected from F) or H) wherein R<sub>14</sub> and R<sub>16</sub> are each independently selected from the group consisting of

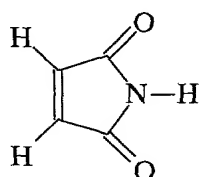


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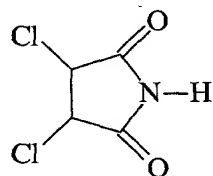
and R<sub>15</sub> is -O- or  $\begin{array}{c} \text{R}_{21} \\ | \\ \text{---N---} \end{array}$ , where R<sub>21</sub> is H, CH<sub>3</sub>, or OH.

9. The angiogenesis inhibitory composition of claim 7 wherein the angiogenesis inhibiting compound is selected from the group consisting of

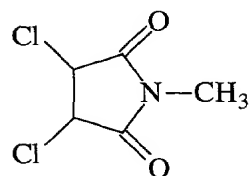
I)



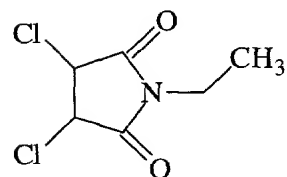
J)



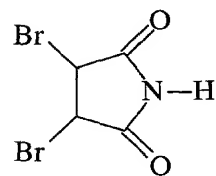
K)



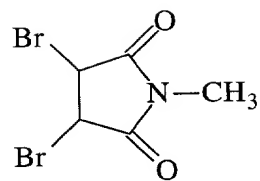
L)



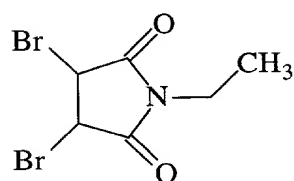
M)



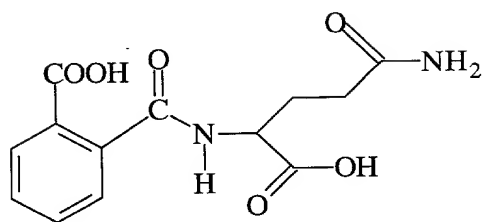
N)



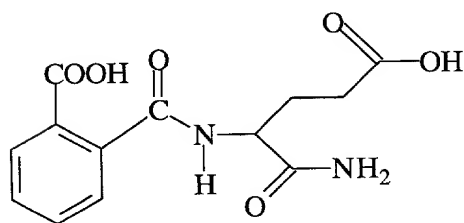
O)



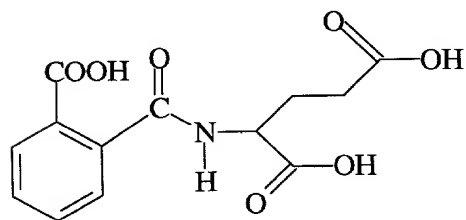
P)



Q)

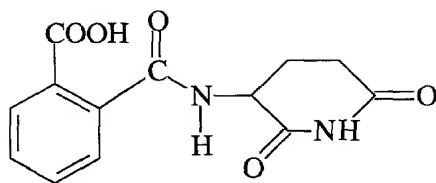


R)



and

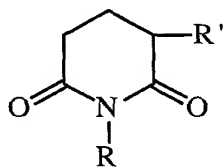
S)



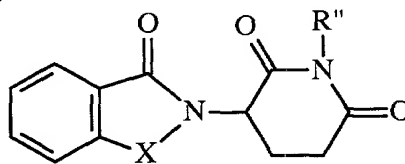
5            10. The angiogenesis inhibitory composition of Claim 7 wherein  
the angiogenesis inhibiting compound is selected from the group  
consisting of thalidomide, metabolites of thalidomide, thalidomide  
analogs, epoxides of thalidomide, hydolysis products thereof, EM-12,  
10            metabolites of EM-12, epoxides of EM-12, hydolysis products thereof,  
EM-138, metabolites of EM-138, epoxides of EM-138, hydolysis  
products thereof, N-phthaloyl-DL-glutamic acid (PGA), N-phthaloyl-  
DL-glutamine anhydride, and mixture thereof.

15            11. The angiogenesis inhibitory composition of Claim 10 wherein  
the inhibiting compound is selected from

(I)



(II)



and

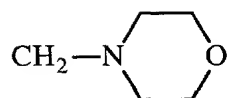
20            wherein  
R is selected from the group consisting of H, (C<sub>1</sub>-C<sub>6</sub>)alkyl, phenyl, and  
benzyl; and

25            R' is selected from the group consisting of phthalimido and succinimido;

wherein

X is CH<sub>2</sub> or C=O; and

R" is H, -CH<sub>2</sub>CH<sub>3</sub>, -C<sub>6</sub>H<sub>5</sub>, -CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, -CH<sub>2</sub>CH=CH<sub>2</sub>, or



5

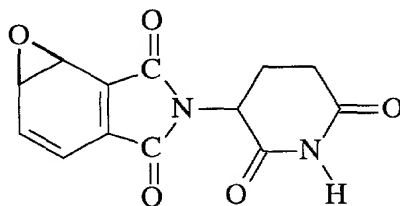
and

(III) hydrolysis products of (II) wherein R" is H and the piperidino ring or both the piperidino and the imido ring are hydrolyzed.

10

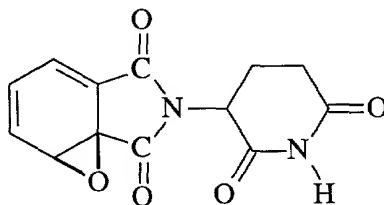
12. The angiogenesis inhibitory composition of Claim 10 wherein the angiogenesis inhibiting compound is selected from the group consisting of

III)



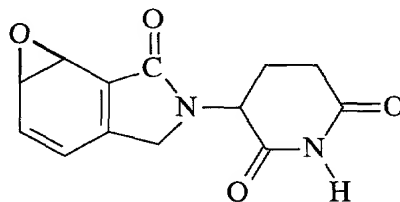
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IV)

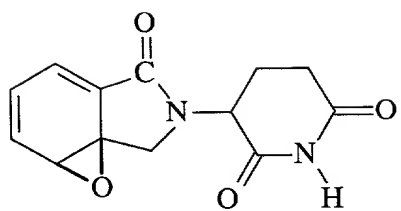


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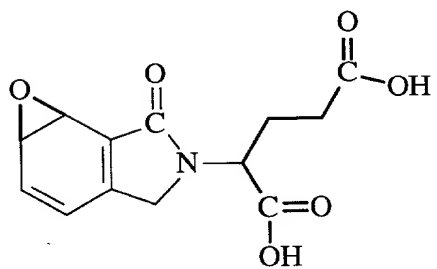
V)



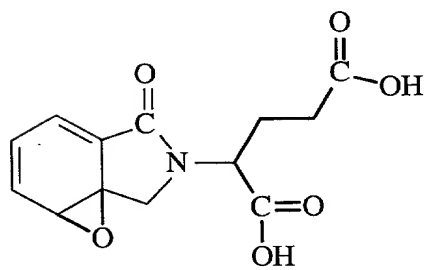
VI)



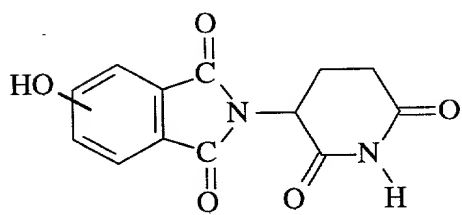
VII)



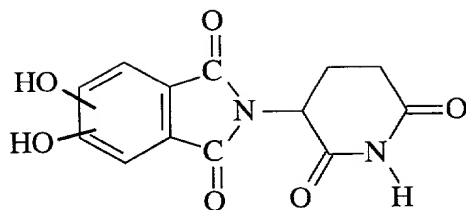
VIII)



IX)

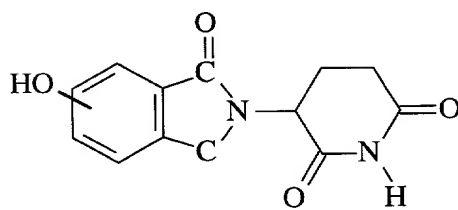


X)

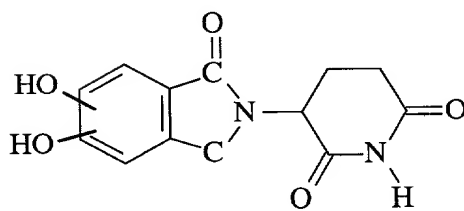


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XI)

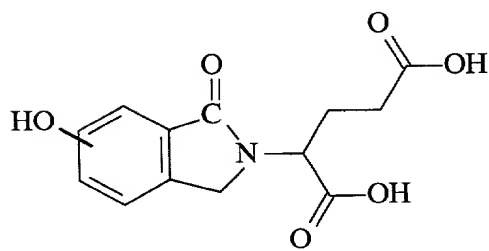


XII)



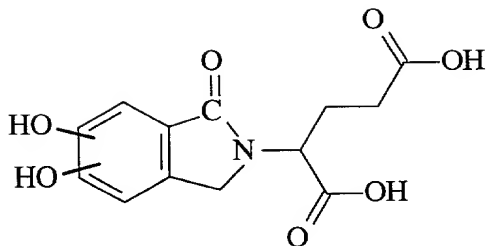
10

XIII)



and

XIV)



5           13. A method for inhibiting angiogenesis in a human or animal comprising administering to the human or animal a composition comprising a nonsteroidal, antiinflammatory drug (NSAID).

10           14. The method of Claim 13 wherein the composition further comprises an angiogenesis inhibiting compound.

15           15. A method for inhibiting angiogenesis in a human or animal comprising administering to the human or animal a composition comprising an angiogenesis inhibiting compound and an antiinflammatory compound.

20           16. A method for treating an angiogenesis dependent disease in a human or animal having such a disease comprising administering to the human or animal a composition comprising a nonsteroidal, antiinflammatory drug (NSAID).

            17. The method of Claim 16 wherein the composition further comprises an angiogenesis inhibiting compound.

25           18. The method of Claim 16 wherein the angiogenesis dependent disease is selected from the group consisting of macular degeneration, diabetic retinopathy, neovascular glaucoma, retrolental fibroplasia, proliferative vitreoretinopathy, solid tumors, blood-borne tumors, leukemia, hemangioma, psoriasis, Kaposi's sarcoma, Crohn's  
30           disease, ulcerative colitis, cancer, retinopathy of prematurity, corneal

graft rejection, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogren's syndrome, acne rosacea, phlyctenulosis, syphilis, *Mycobacteria* infections, lipid degeneration ,  
5 chemical burns, bacterial ulcers, fungal ulcers, *Herpes simplex* infections, *Herpes zoster* infections, Mooren's ulcer, Terrien's marginal degeneration, marginal keratolysis, trauma, rheumatoid arthritis, systemic lupus, polyarteritis, Wegener's sarcoidosis, scleritis, Stevens-Johnson disease, radial keratotomy, corneal graft rejection, sickle cell  
10 anemia, pseudoxanthoma elasticum, pemphigoid, Paget's disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis, chronic vitritis, Lyme's disease, systemic lupus erythematosus, Eales' disease, Behcet's disease, presumed ocular histoplasmosis, Best's disease, myopia, optic pits, Stargardt's disease, pars planitis, chronic retinal  
15 detachment, hyperviscosity syndromes, toxoplasmosis, post-laser complications, and rubeosis.

19. A method for treating an angiogenesis dependent disease in a human or animal having such a disease comprising  
20 administering to the human or animal a composition comprising an angiogenesis inhibiting compound and an antiinflammatory compound.

20. The method of Claim 19 wherein the angiogenesis dependent disease is selected from the group consisting of macular  
25 degeneration, diabetic retinopathy, neovascular glaucoma, retrolental fibroplasia, proliferative vitreoretinopathy, solid tumors, blood-borne tumors, leukemia, hemangioma, psoriasis, Kaposi's sarcoma, Crohn's disease, ulcerative colitis, cancer, retinopathy of prematurity, corneal graft rejection, epidemic keratoconjunctivitis, Vitamin A deficiency,  
30 contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogren's syndrome, acne rosacea, phlyctenulosis, syphilis, *Mycobacteria* infections, lipid degeneration , chemical burns, bacterial ulcers, fungal ulcers, *Herpes simplex* infections, *Herpes zoster* infections, Mooren's ulcer, Terrien's marginal  
35 degeneration, marginal keratolysis, trauma, rheumatoid arthritis,

- systemic lupus, polyarteritis, Wegener's sarcoidosis, scleritis, Stevens-Johnson disease, radial keratotomy, corneal graft rejection, sickle cell anemia, pseudoxanthoma elasticum, pemphigoid, Paget's disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis, chronic vitritis, Lyme's disease, systemic lupus erythematosus, Eales' disease, Behcet's disease, presumed ocular histoplasmosis, Best's disease, myopia, optic pits, Stargardt's disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, post-laser complications, and rubeosis.
- 5

**METHODS AND COMPOSITIONS  
FOR INHIBITION OF ANGIOGENESIS**

**Abstract of the Disclosure**

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The present invention comprises a group of compounds that effectively inhibit angiogenesis. More specifically, thalidomide and various related compounds such as thalidomide precursors, analogs, metabolites and hydrolysis products have been shown to inhibit angiogenesis and to treat disease states resulting from angiogenesis. Additionally, antiinflammatory drugs, such as steroids and NSAIDs can inhibit angiogenesis dependent diseases either alone or in combination with thalidomide and related compounds. Importantly, these compounds can be administered orally.

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Attorney Docket No. 05213-0272

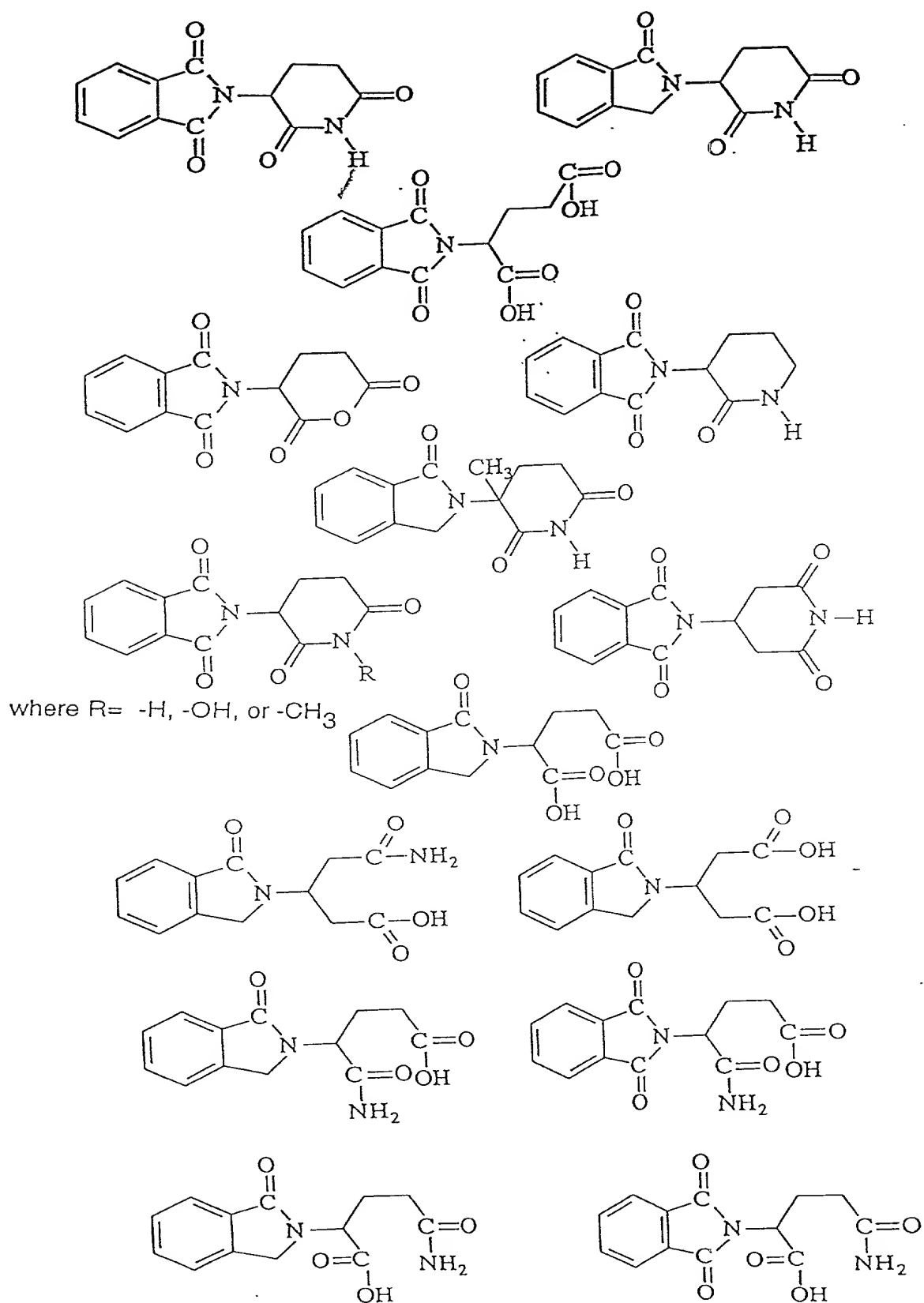


FIGURE 1

Chemical structures of various phthalimide derivatives and their derivatives.

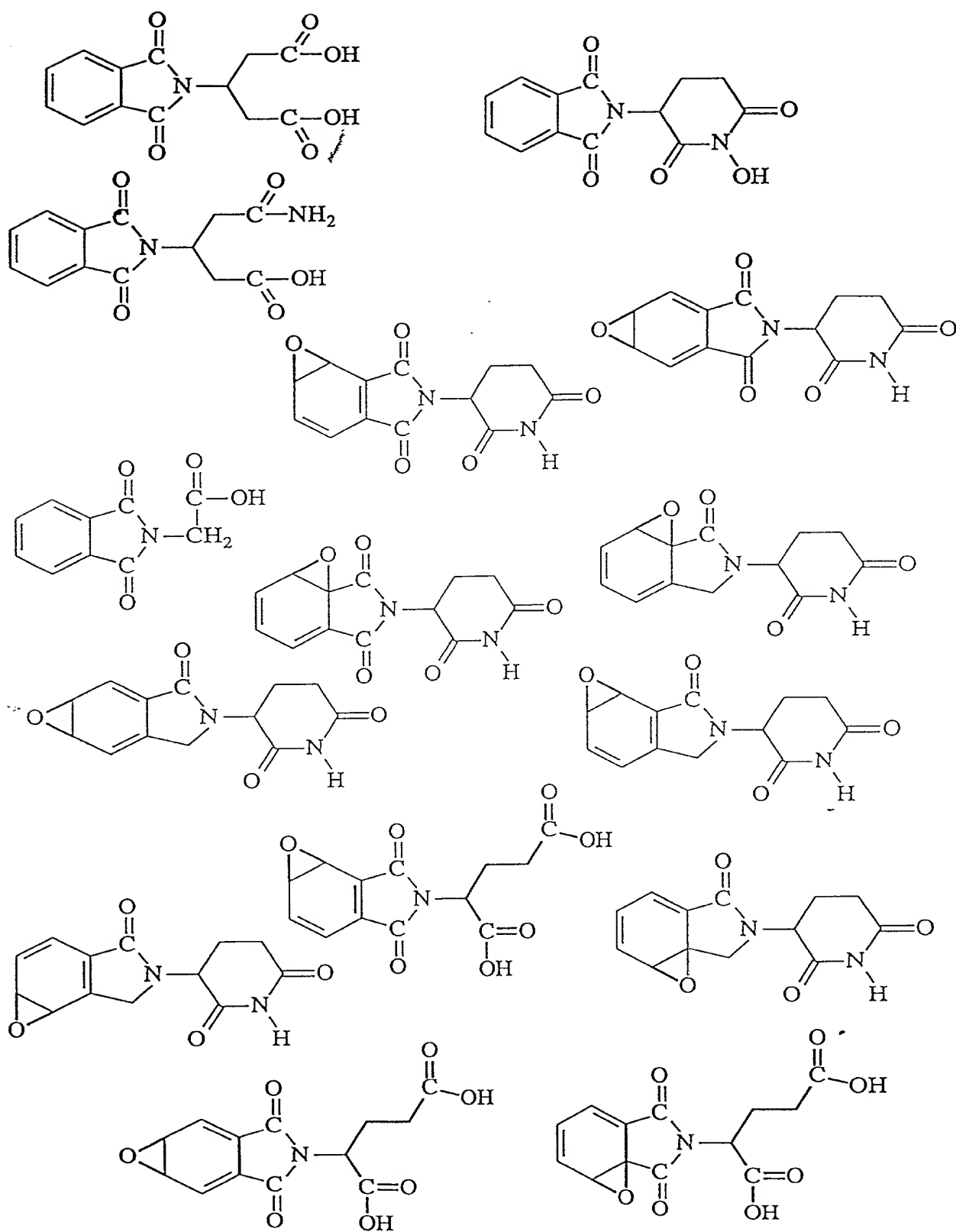


Figure 2

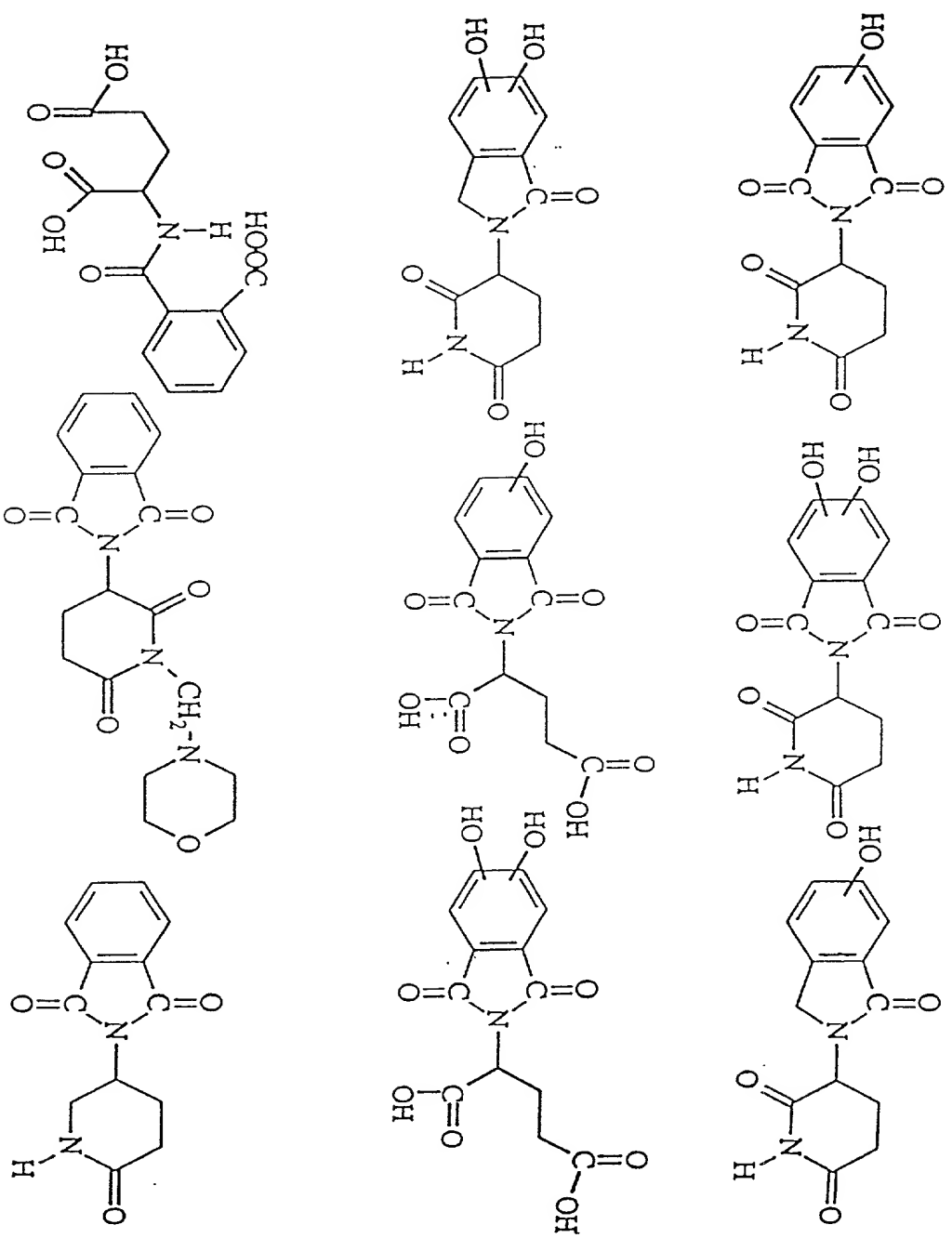


Figure 3

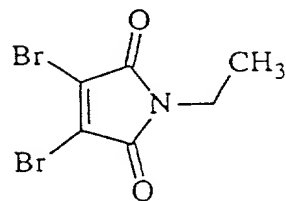
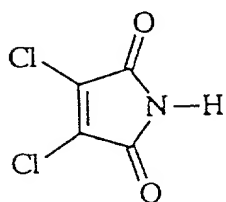
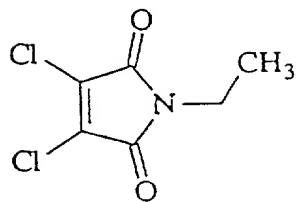
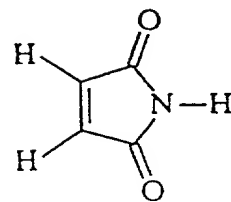
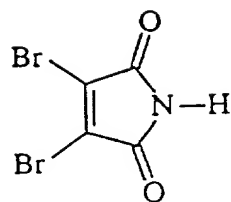
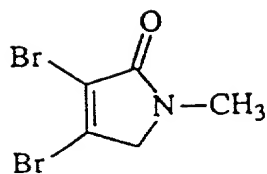
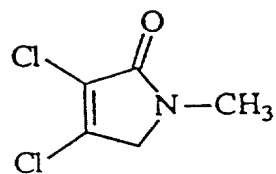


Figure 4

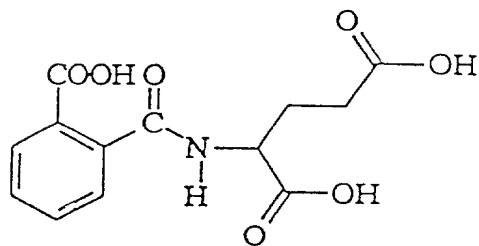
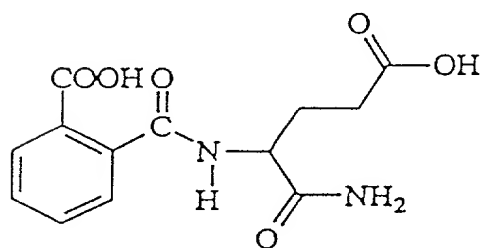
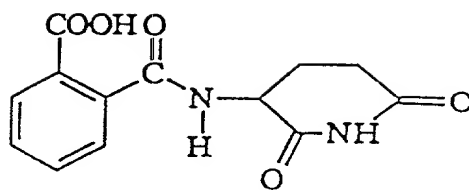
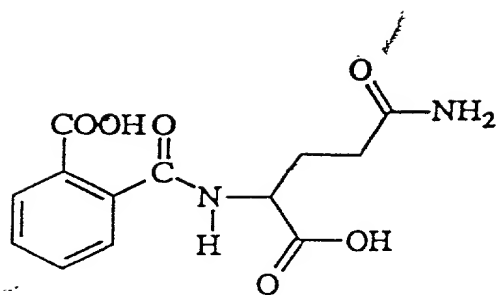


Figure 5

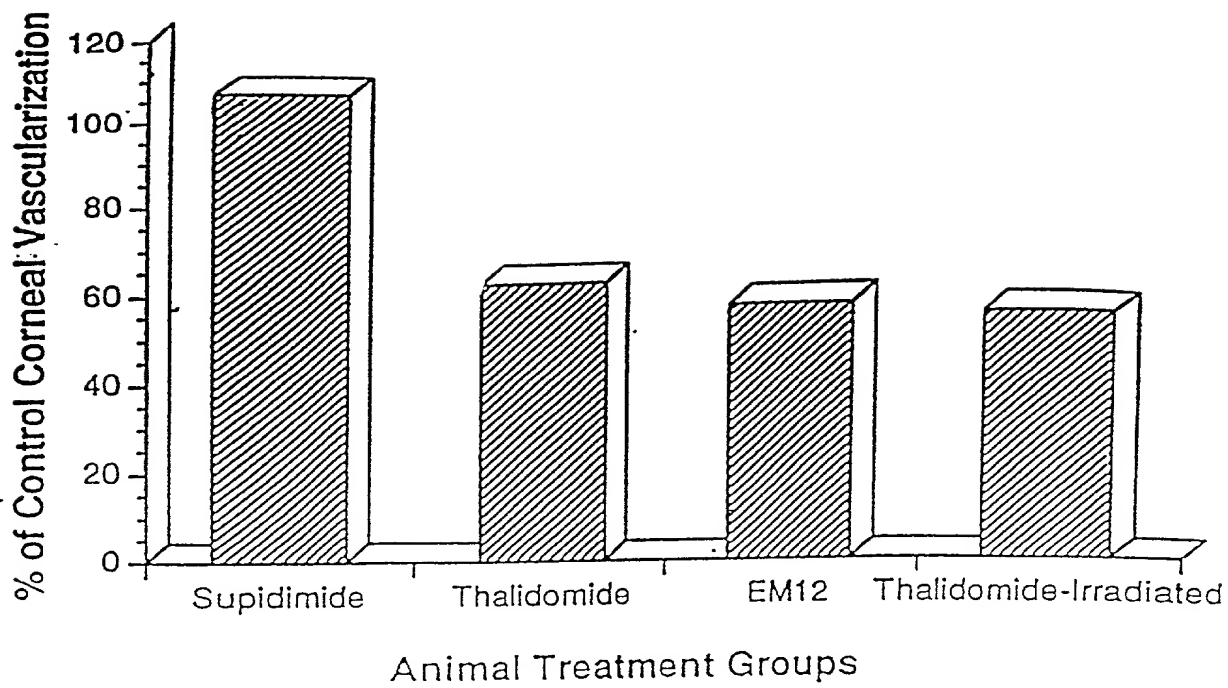


Figure 6

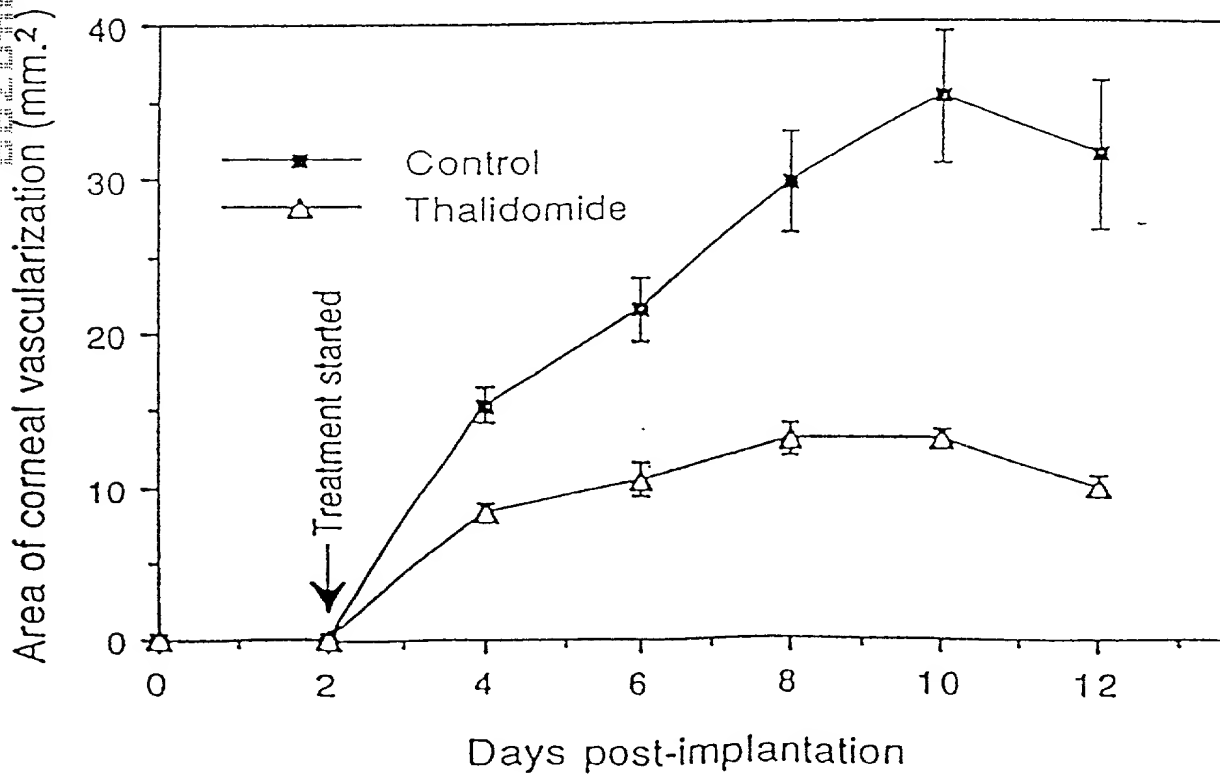


Figure 7

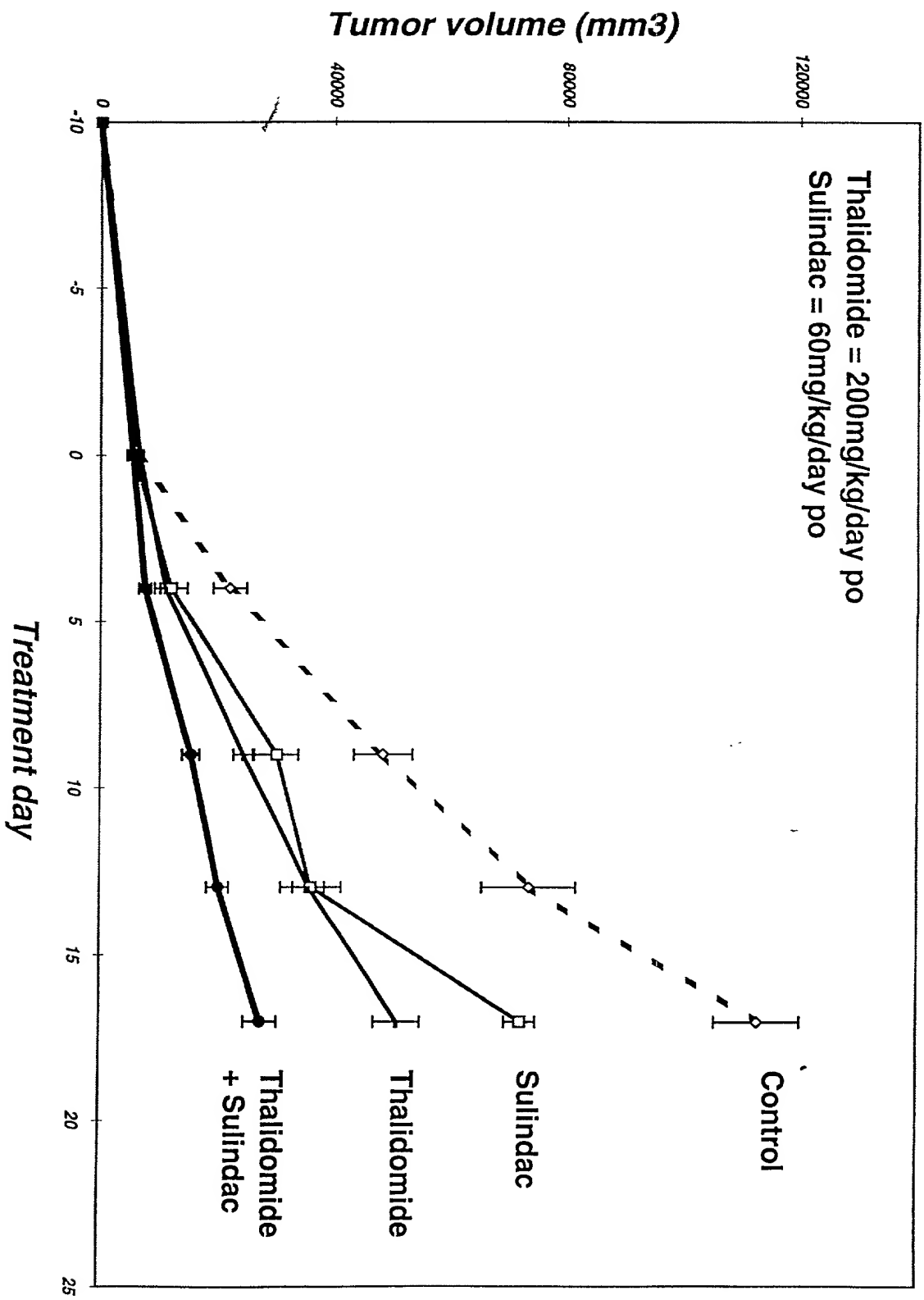


FIGURE 8

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**DECLARATION AND POWER OF ATTORNEY**Attorney's Docket No. **05213-0271**As a below named inventor, I hereby declare that: **Robert J. D'Amato**

My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: **METHODS AND COMPOSITIONS FOR INHIBITION OF ANGIOGENESIS**, the specification of which was filed on **November 3, 1997** as Application No. **08/963,058**.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I do not know and do not believe that the same was ever known or used by others in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to the date of this application. I further state that the invention was not in public use or on sale in the United States of America more than one year prior to the date of this application. *I understand that I have a duty of candor and good faith toward the Patent and Trademark Office*, and I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) of the foreign application(s) for patent or inventor's certificate listed below, and have also identified below any foreign application for patent or inventor's certificate disclosing subject matter in common with the above-identified specification and having a filing date before that of the application on which priority is claimed:

| <u>Application No.</u> | <u>Country</u> | <u>Filing Date</u> | <u>Priority Claimed Under 35 USC §119</u> |
|------------------------|----------------|--------------------|---|
|                        |                |                    | Yes _____ No _____                        |

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

|                   |                         |                   |               |
|-------------------|-------------------------|-------------------|---------------|
| <u>60/028,708</u> | <u>November 5, 1996</u> |                   |               |
| (Application No.) | (Filing Date)           | (Application No.) | (Filing Date) |

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter disclosed and claimed in the present application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

| <u>Application Serial No.</u> | <u>Filing Date</u> | <u>Status: patented, pending, abandoned</u> |
|-------------------------------|--------------------|---|
|-------------------------------|--------------------|---|

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patents issuing thereon.

**POWER OF ATTORNEY:** The following attorneys are hereby appointed to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Anthony B. Askew - 24,154; Roger T. Frost - 22,176; Jeffrey E. Young - 28,490; Robert E. Richards - 29,105; John R. Harris - 30,388; Stephen M. Schaetzel - 31,418; Larry A. Roberts - 31,871; Thomas A. Hodge - 22,602; Charles L. Warner II - 32,320; Gregory T. Gronholm - 32,415; Dale Lischer - 28,438; Peter G. Pappas - 33,205; James Dean Johnson - 31,771; Nora M. Tocups - 35,717; W. Scott Petty - 35,645; Daniel J. Warren - 34,272; Hubert J. Barnhardt III - 36,739; Virginia L. Carron - 37,110; Leona G. Young - 37,266; Jamie L. Greene - 32,467; Holmes J. Hawkins III - 38,913; Mary Anthony Merchant - 39,771; Michael J. Mehrman - 40,086; William L. Warren - 36,714; F. Leslie Bessenger III - 39,108; James A. Witherspoon - 36,723; Brenda M. Ozaki - 40,339; James D. Withers - 40,376; M. Todd Mitchem - 40,731; Gregory S. Smith - 40,819; John J. Snyder - 39,380; Kimberly J. Prior - 41,483; William B. Lafferty - 39,259; Veda V. Christmas - P41,675; William B. Dyer III - P41,725; Theodore M. Green - P41,801; Christopher J. Leonard - P41,940; John E. Whitaker - P42,222; Christos S. Kyriakou - P42,776; John K. McDonald - P42,860; Michael S. Pavento - P42,985.

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**Kimberly J. Prior**

|   |                            |
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| Full name of sole or first inventor: <u>Robert J. D'Amato</u>                             | Citizenship: <u>U.S.A.</u> |
| Inventor's signature: <u>[Signature]</u>  | Date: <u>3/9/98</u>        |
| Residence and Post Office Address: <u>65 Huron Avenue, Cambridge, Massachusetts 02138</u> |                            |